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STREAK OR WINTER BLIGHT OF TOMATO IN QUEBEC

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WITH THREE FIGURES IN THE TEXT

An outbreak of streak, stripe, or winter blight of tomato, which was characterised by its sudden onset and virulent nature and which resulted in a loss of one-third of the crop, occurred on the tomato crop in the commercial greenhouses at Macdonald College in the spring of 1923.

Diseases of the tomato with apparently identical symptoms have been reported under a variety of names from widely separated parts of the world, and there seems to be considerable difference of opinion among investigators regarding their etiology. In view of this and the increasing importance of the greenhouse tomato industry in Quebec, a study of the disease under both field and greenhouse conditions was undertaken.

PREVIOUS INVESTIGATIONS

According to Orton and McKinney (16) the first mention of such a disease was by Lodeman in 1892, when he reported the occurrence of a blight of winter tomatoes in the greenhouses at Ithaca, N. Y., during the winter of 1890–91. In the same year Bailey (3) gave a fuller account of the trouble, to which he ascribed the name winter blight. Its primary cause remained undetermined, though the view was held that it was bacterial. Four years later Selby (19) described a blight of forced tomatoes in Ohio, with symptoms similar to those given by Bailey. Selby noticed that the youngest leaves showed earliest symptoms of the disease, all plants being liable to attack regardless of their thriftiness, and that most of the plants with diseased foliage had clean fruit. Winter blight of the tomato was reported from Pennsylvania in 1915, by Orton and McKinney (16), but the disease had been under investigation there for

[1] The writer is indebted to Dr. B. T. Dickson for many helpful suggestions, and for his kindness in affording field and greenhouse facilities.

some five years or more. They isolated in pure culture, light yellow and orange-brown, bacterial organisms from the lesions of diseased plants, but all attempts to produce infection by artificial inoculation were unsuccessful. Jackson (10) reported winter blight as having first appeared in the Indiana Station greenhouses in the winter of 1915. In 1916 the disease was first reported from Canada by Howitt and Stone (9). Repeated trials on their part to isolate a causal organism from the diseased tissues proved futile. These investigators are of the opinion that the disease is not seed transmitted, and carefully conducted experiments led them to believe that the cause of the trouble lies in the soil. In 1918–19, what appears to be the same trouble was reported from South Australia by Osborne (17), and

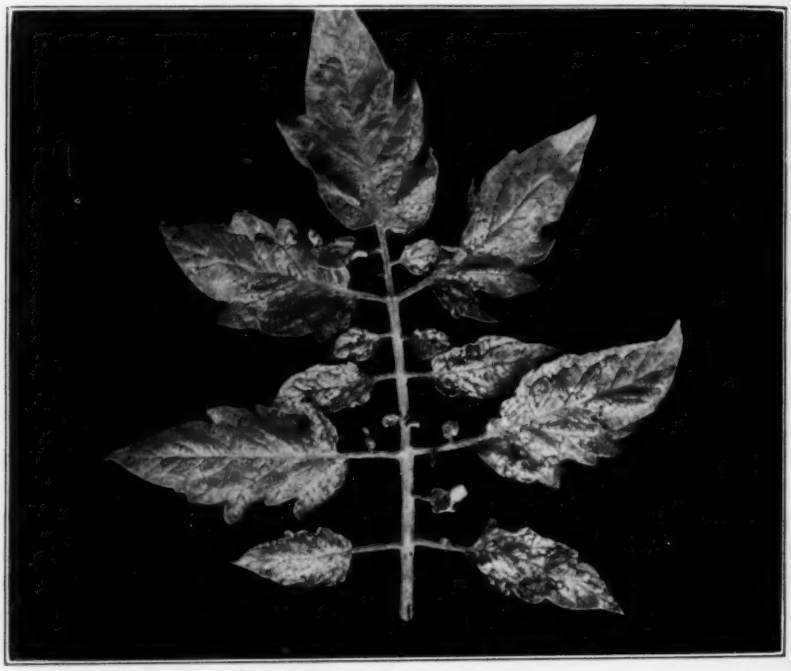

FIG. 1. A typical streak tomato leaf showing irregular necrotic spots.

from Victoria by Brittlebank (4). Two years later, 1921, Hamblin (8), from New South Wales, discussed a "spotted wilt" of tomato, which is probably none other than streak. Taylor (21) has reported it from New Zealand. In 1919 Paine and Bewley (18) published an account of a disease of tomato, in England and the Channel Islands, with symptoms apparently identical with those of streak or winter blight as described on this continent. These investigators isolated *Bacillus lathyri* Manns and

Taubenhaus, from diseased tissues, and with it they could bring about artificial infection of rapidly-growing tomato plants. Gardner and Kendrick (7), in a bulletin on tomato mosaic published in 1922, are of the opinion that the diseases described by Bailey of New York and Selby of Ohio, as well as those reported from Pennsylvania and Ontario, are mosaic in its most destructive form. Dr. B. T. Dickson, who reported the disease as being present in Quebec in 1923, inclined to the view that it is a mosaic in which necrosis occurs under certain environmental conditions. In a paper delivered before the Canadian Phytopathological Society in 1924, Stone (20) maintained that he could "produce the disease at will by using in excess fertilizers very rich in nitrogen, such as barnyard manure and ammonia or nitrates," and that the cause of the trouble is in the malnutrition of the plants.

From the foregoing historical sketch it is seen that three views are held regarding the etiology of streak or winter blight by workers on the problem, *viz:* (*a*) that the disease is caused by bacteria; (*b*) that it is of a purely non-parasitic nature, being brought about by malnutrition and improper management of environmental conditions in the greenhouse; (*c*) that the disease is a manifestation of mosaic in a most severe form.

SUMMARY OF REPORTS ON THE ECONOMIC IMPORTANCE OF TOMATO STREAK

Authority	Locality	Estimate of losses
Orton and McKinney (16)	Pennsylvania	5 per cent a fair average in greenhouses
Howitt and Stone (9)	Ontario	10 per cent in two greenhouses near Hamilton
Chupp (Correspondence)	New York	1 to 3 per cent in greenhouses
Thomas (do)	Ohio	Sporadic: 60 per cent in some greenhouses
Osmun (do)	Massachusetts	Some greenhouse crops almost ruined
Dickson (Observation)	Quebec	1923. One third of the greenhouse crop
Poole	New Jersey	Streak and filiform conditions caused 95 per cent loss in several greenhouses. (Plant Dis. Reporter Suppl. 25: 61. 1923.)
The writer's observations	Quebec	1924. 2 per cent in one greenhouse crop

GEOGRAPHICAL DISTRIBUTION

In North America the disease is probably most serious in Pennsylvania, New York, New Jersey, Northeastern Ohio, and Southern Ontario; and as reported to "The Plant Disease Survey" and according to Orton (15), it is also found in Indiana, Massachusetts, Michigan, Kansas, California and Washington. It has been observed in greenhouses in Maryland.

In appears to be more prevalent in the greenhouse than in the field, and on the autumn tomato crop than on the spring crop.

SYMPTOMS

An attempt to describe a set of clear-cut symptoms of the streak disease of tomato would necessarily be inadequate because of the various forms which the disease takes, depending apparently upon the succulence and age of the plant, as well as upon the temperature, illumination and other environmental factors.

In Quebec the disease usually is first noticed on tomato plants about the same time that the first blossom truss appears. Necrotic streaks on stems and spots on leaves occur in a zone about a foot in extent two-thirds up the plant. In this region irregular yellow spots appear on the leaves and subsequently turn brown and finally quite dark, being surrounded by yellowish areas (Fig. 1). Complete wilting of the leaves in this zone often results. Should optimum conditions for the disease continue to prevail, complete necrosis with wilt and drying out will occur. If, on the other hand, a slower growth is induced, or if the plants are hardened off slightly, then the upper growth becomes spindling and distorted; the leaves small and ruffled, weak, crinkled and curled downwards with numerous yellowed and minute necrotic areas. At this stage these plants invariably show marked symptoms of mosaic as well. On looking down the rows between the greenhouse beds, the spindling growth of the upper part of the plant is very prominent and once seen is unmistakable thereafter. Infected plants usually linger on in this condition for several weeks, and may even produce a moderate crop of fruit, only part of which will be blemished by spots; but even in this condition these plants serve as sources of infection for healthy plants.

Immediately below the diseased zone there are on the average 8 to 10 strong, apparently healthy, mature lower leaves. These seldom have lesions, but the young shoots which arise from the axils of these leaves usually have typical symptoms.

The brown linear lesions on the stem vary from one millimeter to several centimeters in length (Fig. 2). They enlarge and often coalesce, although this is a variable condition depending on the duration of optimum conditions for the disease. The lesions on the petiole are often so large that

they cause it to shrivel, turn brown and dry out with the leaflets hanging dead and dry.

The lesions on the flower-stalk and sepals are small and not very elongated. Similar spots occur on petals and stamens.

All the fruit on a diseased plant may be entirely free from outward blemishes or a portion of them may show lesions. In the same truss some fruits may be spotted and others apparently healthy; also, some trusses of fruit may show signs of the disease while others on the same plant may

FIG. 2. Brown, slightly sunken, streak lesions on stems of tomato. Coalescence is seen at "a."

be normal. Plants have been found with much of their fruit diseased but typical streak lesions on stems and leaves were entirely absent; these plants, however, had definite symptoms of mosaic in varying degrees of severity. Symptoms on the fruit differ according to the variety of tomato. For the most part, irregular, raised, brown, greasy patches with no definite arrangement occur on the fruit (Fig. 3.) These frequently coalesce and form

quite large sunken patches in which cracks or slits soon develop owing to their drying out and the subsequent growth of the fruit. Severely affected fruits often are deformed.

An examination of the roots, both externally and internally, reveals no apparent divergence from the roots of normal plants, except that perhaps the root system is reduced somewhat.

At Macdonald College, plants affected with streak undoubtedly had mosaic. In many cases the mosaic symptoms were not clearly apparent at first; but, providing the plants did not die forthwith from the streak, the characteristic symptoms of mosaic always developed later, and usually in a most severe form.

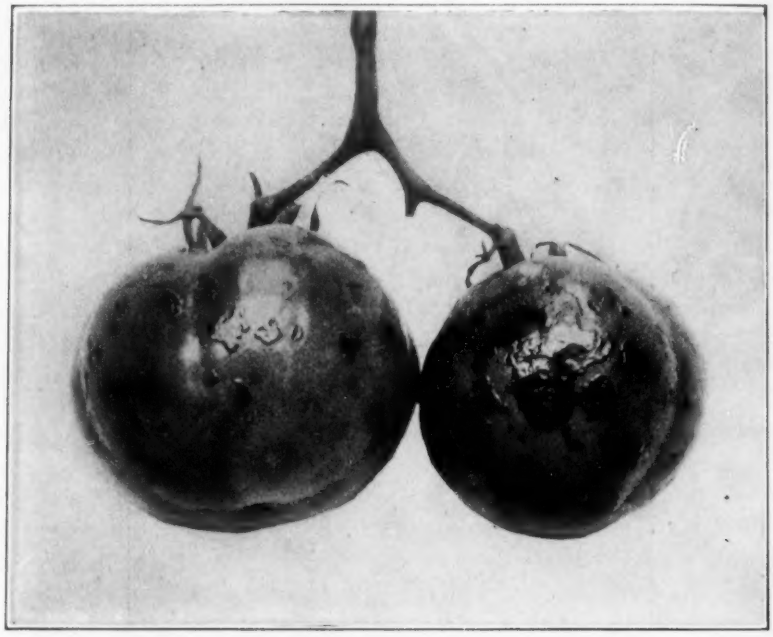

FIG. 3. Raised, brown, greasy patches on fruit of Livingstone Globe tomato. The older lesions are sunken and cracked because of desiccation of the cells beneath.

Internal symptoms.—Cross sections of diseased stems show that usually the lesions are merely superficial, but in some the necrosis extends well into the cortex, and in others the pith is invaded as well. The vascular elements do not show any definite necrosis.

All the tissues of the leaf, except the vascular bundles, become necrotic. There is a shrivelling and drying up of the lamina in affected areas.

The fruit spots involve only a few layers of cells, but occasionally minute patches of diseased tissue are noticed here and there within the fruit.

EXPERIMENTAL STUDIES

The experimental investigations upon which this paper is based were conducted under field conditions during the summers of 1924 and 1925, and in the hothouse during the intervening winter. Space did not permit of dealing with large numbers of plants and, to overcome this difficulty, the experiments were carried out on a small number of plants on three, four, or more, separate occasions. Further, by conducting the experiments both in the greenhouse and in the field, results of comparative value from an environmental standpoint have been added. Therefore the writer thinks that the paucity in the number of plants was overcome, and that the results are actualities.

All field inoculations were made just before dusk and on rainy evenings whenever possible. If no rain had fallen, the plants were thoroughly drenched with water before inoculation. Inoculations in the greenhouse were performed at any time during the day.

The soil in the experimental field plots was a loam which had received a dressing of farmyard manure the previous autumn; that used in pots in the greenhouse was a rich sod soil, to which farmyard manure was added.

In all experiments conducted in the greenhouse care was given to the control of aphids and white flies.

Bacterial Isolations and Inoculations

Numerous attempts to isolate a causal organism from diseased tissue were made. Most of these yielded no growth whatever, but a small percentage yielded a yellowish bacterial growth, which on plating out gave numerous yellow colonies. What appeared to be a similar organism was isolated from superficial and internal lesions both from the fruit and from the stem. Only occasionally could it be obtained from diseased leaves. This yellow organism is so irregularly found, however, that it can hardly be said to be constantly associated with streak.

Fresh bouillon cultures of the various strains of the yellow organism obtained from fruit, stem, and leaf respectively, of diseased plants both from the field and under glass, were repeatedly sprayed on young healthy tomato plants, some of which were wounded beforehand by rubbing the leaves gently between the finger and thumb. Similarly, cultures on nutrient agar were pricked into the stems of other plants and the wound covered with tin foil. After all such inoculations the plants were covered with large bell jars for two or three days. Sometimes a piece of sterile

cotton which had been soaked in a nutrient solution of the organism was wrapped around the needle-pricked host part and then covered with tin foil. All precautions to keep the plants from drying out were observed. On no single occasion under controlled conditions did streak develop as a result.

Through the kindness of Dr. Sydney G. Paine, Imperial College of Science and Technology, London, the author obtained a culture of *Bacillus lathyri*, which was isolated from clover, but which is identical in its physiological behavior with the strain from tomato, according to Dr. Paine. All artificial inoculations with this organism into rapidly-growing, healthy tomato plants failed to produce typical streak symptoms after a month and a half. This experiment was tried repeatedly. The fact that this organism had been growing in culture for nearly three months when the inoculations were made possibly might account for its loss in virulence.

A series of preliminary comparative cultural studies of strains of the yellow organism isolated from streak tomato plants with the strain of *Bacillus lathyri* obtained from Dr. Paine were carried out. These tests point strongly to the fact that the organisms are identical, although further tests will have to be carried out before such can be stated definitely. In all the reactions observed the results secured with the different strains showed no more variation than is common among strains of the same species.

Preliminary Experiments

A preliminary series of inoculation experiments in which both tissue and juice of streak tomato plants were inoculated into healthy tomato plants revealed the fact that infection may be brought about both by rubbing the leaves with diseased tissue and by inserting it into the stem, or in any way in which the extracted juice of diseased plants is brought into contact with that of the healthy plant. Thereafter all artificial inoculations were made either by rubbing the leaves of healthy plants with a small amount of crushed and macerated tissue or with the expressed juice of streak plants, or by pricking in such material with a sterile needle after first placing it on the plant.

Of 57 plants in these early studies so inoculated, over 85 per cent developed streak and mosaic, 8 to 10 per cent contracted mosaic only, and 1 to 2 per cent showed no disease symptoms. Only very occasionally was it difficult to decide to which group a plant belonged. Plants of all ages, and consequently of varying degrees of thriftiness, were thus experimented with; but the juices from streak plants were of such a virile and infectious nature that the disease was almost invariably produced, although the incubation period varied with the plant. In the field, however, on young plants growing under ordinary summer conditions, the incubation period

was invariably 12 days. Under greenhouse conditions with varying light no such regularity obtained during the winter months; but here by far the greater number of plants showed their first lesions in from 11 to 18 days. All control plants, inoculated with macerated tissue from healthy plants, remained healthy.

Systemic Nature of the Disease

As has already been mentioned under symptoms of the disease, there are many mature leaves on the lower portion of diseased plants, which from macroscopic examinations appear to be absolutely free from all malformations, lesions or discolorations of any sort. Several such leaves were carefully removed from diseased plants, and after immersing them in mercuric chloride (1:1000) for one to two minutes, they were washed with sterile water and crushed in a mortar. Healthy tomato plants were then inoculated with this pulp in the usual way. This experiment was repeated four times and in each case streak developed as a result.

An analogous experiment was conducted with clean, unspotted fruit, apparently free from any symptoms of the disease whatsoever, found on otherwise diseased plants. Such fruit also proved to be infectious.

It may be gathered from these experiments that while there are certain mature leaves and fruit which appear perfectly normal, they still serve as carriers of infection for the developing shoots of the same plant and as sources of inoculum for healthy plants. It shows further that streak is a disease which is visible in comparatively nascent tissue only, though all parts of the plant above ground carry the infective principle.

Whether the roots of streak plants are also carriers of infection still requires to be determined.

Nature and Properties of Streak Tomato Juice

The effect of filtration on streak juice.—Expressed juices from leaves, stems and fruit of streak tomato plants were passed through sterile Chamberland F filters into sterile flasks. Under aseptic conditions this filtered juice was put into sterile test tubes from which it could be used at once as a source of inoculum, or kept until required.

Stained preparations of this filtered juice were examined carefully for any microorganisms. As a further check on the freedom of the filtered juice from bacteria, nutrient agar slopes and nutrient broth tubes were also inoculated in duplicate, and incubated at 22° to 26°C. No growth resulted from such filtered juice.

A portion of the unfiltered juice was always saved and inoculated into healthy tomato plants so as to serve as a check on the viability of the streak juice before passage through the filter. The results of the inoculations are given in table 1.

TABLE 1.—*Results of inoculating healthy tomato plants with expressed streak tomato juice which had passed through a Chamberland F filter*

No. of tomato plants inoculated	Inoculum	Results		
		Streak	Mosaic only	No positive results
Greenhouse 1924				
11	Filtered streak juice (bacteria free), plants wounded	7	4	0
1	Filtered juice sprayed on plant (without wounding)	0	0	1
1	Filtered juice poured on soil	0	0	1
Field 1924 and 1925				
11	Filtered streak juice (bacteria free), plants wounded	11	0	0

In every case where streak developed the incubation period varied from 11 to 15 days. The results show conclusively that streak of tomato in Quebec belongs to the filterable-virus group of plant diseases.

The effect of high temperature on the power of infection of streak juice.—This experiment was carried out with a view to ascertaining whether the thermal death point of the infective agent lay in the range of the thermal death point of bacterial plant pathogenes or in that of the filterable viruses.

Ten cubic centimeters of the expressed juices from diseased plants were placed in test tubes with a pipette, care being taken not to allow any juice

TABLE 2.—*The effect of heat on the infectivity of the expressed juice from streak tomatoes*

No. of tomato plants inoculated	Treatment of streak juice used for inoculation	Results		
		Streak	Mosaic only	No positive results
1	45° C. for 10 min.	1 (11 days)	0	0
1	55° C. do	1 (15 days)	0	0
1	65° C. do	1 (13 days)	0	0
5	70° C. do	3 (12–14 days)	1	1
1	75° C. do	1 (15 days)	0	0
2	80° C. do	1 (13 days)	0	1
2	90° C. do	0	0	2

to get on to the sides of the tubes. These tubes were then placed in water held at various temperatures for 10 minutes and cooled under the tap immediately on removal. Inoculations were then performed in the usual way.

To serve as a check on the infective nature of the heated juices, plants were inoculated with unheated juice at the same time that the inoculations with the treated juices were made.

The results of these experiments show that expressed streak tomato juices heated 20° C., or more, above the thermal death point of the majority of bacterial plant pathogenes (*Bacillus lathyri* T.D.P. 50° C.) still retain their infective powers. Also, the exact temperature to which they may be heated without losing this power seems to vary with the source of the juice. This peculiar characteristic has been demonstrated by Allard (1) to be a property of tobacco mosaic, the thermal death point of which he found to vary from 80° to 90° C. for 10 minutes exposure. Doolittle (6) has shown that for cucumber mosaic the thermal death point is about 70° C.

The infective agent for tomato streak, therefore, has a thermal death point lying within the same range as that of other so-called virus diseases.

The effect of drying on streak tomato tissue.—Portions of diseased plants were air-dried at room temperature until thoroughly dry. This material was then ground to a fine powder by passing through a meat chopper, and stored in glass-stoppered bottles until required. For inoculation purposes a water-extract was prepared by soaking some of the fine powder in sterile water over-night. Diseased material which had been stored for two, three, and nine months was used. In no single case did streak develop, but definite mosaic, usually of a very severe nature, invariably was produced instead.

TABLE 3.—*Results of inoculating healthy tomato plants with streak juices after treatment with various strengths of mercuric chloride solution*

No. of plants inoculated	Inoculum		Length of treatment in hours	
	Treated streak juice (Gms. $HgCl_2$: c.c. streak juice)			
1	1	500	2.5	Healthy
1	1	500	24	do
1	1	500	48	do
1	1	800	2.5	Streak (15 days)
1	1	800	24	Healthy
1	1	800	48	do
1	1	1000	48	Streak (15 days)
1	Untreated streak juice (check)			Streak (12 days)

The effect of various chemicals on streak juice.—Mercuric chloride dissolved in water was added to streak tomato juice so that the resulting solution would be of the required dilution. Five cc. portions were drawn off from time to time for experimental purposes as required, and at time of inoculation this treated juice was diluted with an equal amount of sterile water so as to prevent injury to the tomato plants from the mercuric chloride itself. The inoculations were made by crushing the leaf with the finger and thumb and rubbing in the inoculum. The results are given in table 3.

One cc. of the inoculum was plated out in each case at time of inoculation but no growth whatever appeared on the plates at any time. From the above it appears that the streak juice can withstand mercuric chloride in concentrations fatal to bacteria.

Effect of Various Fertilizers in Relation to the Disease

From observations made on outbreaks in the college greenhouses, there appeared to be some connection between the vegetative growth of the plant and the incidence of the disease. Paine and Bewley (18) found that the application of potash largely counteracted the susceptibility of tomato plants to the stripe disease which they investigated; and Stone (20) recommends in his control suggestions that both acid phosphate and potassium sulphate be added.

TABLE 4.—*The influence of fertilizers on the incidence of the disease*

No. of tomato plants inoculated	Inoculum	Fertilizer treatment	No. of days after which streak developed
Field 1924			
5	Virile juices of streak tomato plants in all cases	No treatment	12[a]
5		Farmyard manure—heavy application	12
5		Acid phosphate, 3 oz. per plant	12
5		Sodium nitrate, 1 oz. per plant	12
5		Potassium sulphate ¼ oz. per plant	12
5		No treatment	12
Greenhouse			
2		Sodium nitrate	12 each
1		Manure + nitrate	14
2		Potassium sulphate	12 and 13 respectively
2		Acid phosphate	13 and 15
1		Acid phosphate + potassium sulphate	12

[a] One plant developed severe mosaic only.

Accordingly one series of manurial treatments was conducted in the field in 1924 and another in pots in the greenhouse during the winter. The results of both series of experiments are given in table 4.

In the field the fertilizer was applied one week before inoculation. In the greenhouse experiment the fertilizer applications were at intervals of one week beginning when the tomato seedlings were four inches high. The amount applied was not measured in this case, but the doses were very heavy. The inoculations were made between the second and third applications.

It may be gathered from these results that soil treatments alone cannot prevent infection when the plants are artificially inoculated with viable streak tomato juices. The disease appears after the usual incubation period regardless of fertilizer treatment, but the severity of the disease is slightly more pronounced on plants with the most succulent growth. Providing other environmental conditions are favorable for the development of streak, the plants will not completely "grow out of" the disease, even if the various fertilizer treatments are continued. The addition of chemical fertilizers alone, threfore, will not control streak; but, insofar as it prevents over-succulence in growth, it will help to ward off the disease, especially if other environmental conditions are carefully attended to.

Seed Transmission

Since tomato fruits are sometimes very severely affected, though for the most part only superficially, it might be expected that the disease possibly is transmitted by the seed. Orton (15) lists tomato streak among the seed-borne diseases, and attributes its recent appearance in widely separated parts of the world to this fact.

On three different occasions seed from severely spotted fruit was removed from the pulp, dried and then sown. As streak failed to develop in any of the plants from such seed, it must be inferred that tomato streak is not usually seed transmitted. The fact that necrotic areas within the fruit often line the ovary wall, however, suggests the possibility of occasional seed transmission.

Insect Transmission

Transmission of streak by aphids was readily demonstrated under controlled conditions by allowing aphids, ordinarily found on tomato, to suck the juices of streak tomato plants for a few days and then placing them carefully with a camel-hair brush on vigorously-growing healthy tomato plants. In over 75 per cent of the plants streak symptoms appeared after the usual incubation period.

Three attempts to ascertain whether white flies transmitted the disease were made, but in every case the plants remained healthy.

Developmental Relations of Streak

Temperature relations.—Healthy plants inoculated with streak tomato juice and placed in a hothouse maintained at a temperature of 65° to 75° F. during the day, developed symptoms of streak in 11 to 14 days as a rule. But it was noticed that similarly inoculated plants in the house maintained at 55° to 65° F. took a longer time for symptoms to appear. In several such cases where symptoms were not evident in three weeks, the plants were removed to the warmer greenhouses and in one to three days typical streak lesions appeared.

These results are only indicative since it was difficult to keep the greenhouse at a constant temperature from day to night; but they show, nevertheless, that higher greenhouse temperatures are more favorable to the devlopment of streak than the lower temperatures. Carefully controlled temperature-chamber experiments would probably produce interesting results.

On the other hand, if streak tomato plants growing under conditions suitable for streak development are hardened off by subjecting them to lower temperatures, the new shoots which appear subsequently will show little or no indications of streak.

An endeavor to induce the disease by manipulation of environmental factors.—Several series of rapidly-growing tomato seedlings were placed in soil consisting of one part of well-rotted manure and three parts of sod soil. Both healthy seedlings and young mosaic plants were included in the experiment. Small amounts of sodium nitrate were added to some of these plants about every seven days; to others, ammonium sulphate was applied. All the plants were supplied abundantly with water and kept at the higher greenhouse temperatures which were known to be favorable to the development of streak.

All attempts to induce streak in these plants by forcing their growth with nitrogenous fertilizers, by avoiding the use of acid phosphate and potash, and by over-watering and then allowing to become dry, produced negative results. Contrary to this, Dr. Stone, in a paper presented before the Canadian Phytopathological Society at Ottawa in December, 1924, maintains that he can produce the disease at will by using excess nitrogenous fertilizers, but in no case does he mention mosaic symptoms on these plants.

Johnson (11 and 12) and Tomkins (22) have shown that if mosaic potato or tobacco plants are subjected to temperatures unfavorable to the development of mosaic, then the symptoms will disappear; but when these plants are placed under optimum temperatures for their respective type of mosaic, the mosaic symptoms will return. From the study of the temperature relations elsewhere outlined, the same appears to be true for tomato streak. It is possible, therefore, for tomato plants to be growing

under conditions which would mask all streak symptoms and yet themselves be carriers of the agent of streak. Atanasoff (2) has shown that there are varieties of potatoes which serve as carriers of potato streak, but which do not themselves show any symptoms.

Cross Inoculations

Tobacco. Preliminary experiments showed that when healthy Resistant White Burley tobacco plants were inoculated with macerated tissue or extracted juice of streak tomato, either by rubbing the leaves or by pricking the plant through the applied juice, a mosaic of a very serious nature developed almost without failure in 10–14 days. Filtration through Chamberland F filters did not destroy the infectivity of this juice. The expressed juices from these infected tobacco plants were inoculated into healthy tomato plants both in the field and in the greenhouse. Table 5 contains the summarized results.

TABLE 5.—*Results of inoculating healthy tomato plants with juice of mosaic tobacco developed from tomato streak*

No. plants inoculated	Host	Source of inoculum	Streak	Mosaic only	No positive results
16	Greenhouse tomato plants	Juice of mosaic tobacco developed from streak tomato	11	3	2
9	Field tomato plants	Do	6	3	0
2	Greenhouse tomato plants	Do Juice passed through a Chamberland F filter	1	0	1

From such inoculations typical streak, followed later by mosaic, was produced in 69 per cent of the tomato plants. Clinton (5, p. 373) describes a bacterial-like burn produced in tomato from mosaic or calicoed tobacco. From Plate XXIX in this reference in which leaves and stems of this tomato plant having burn are shown, it would appear that this so-called burn is identical with streak or winter blight.

Other hosts. The results of similar cross inoculations, using ground cherry (*Physalis heterophylla*), pepper (*Capsicum annuum*), and eggplant (*Solanum melongena*), as intermediate hosts, indicate strongly that these Solanaceous plants may sometimes act as carriers of streak in a way similar to tobacco in the experiment outlined above. Further work on this phase of the problem is necessary.

Effect of Inoculating Tomato with Combination Viruses

The fact that mosaic is invariably present on streak tomato plants, and that streak material which has been dried for months produces mosaic only, when inoculated into tomato, suggested that there might be some other factor, possibly another infective agent which, together with tomato mosaic, produced the disease known as streak. This other factor presumably could not withstand prolonged drying. To show that true streak was not a severe manifestation of tomato mosaic only, the author subjected tomato plants showing pronounced mosaic symptoms to those conditions which had been shown to favor the development of streak most, and in no single case did true streak symptoms appear.

The publication of Johnson's findings (13) substantiated this view. This investigator, by inoculating a combination of potato mosaic and tobacco mosaic virus into tobacco and tomato, obtained leaf spots and blotches which sometimes killed entire leaves. This suggested the possibility that the other factor for which the present author had been searching was potato mosaic.

Tomato mosaic and potato mosaic combined. Juices were expressed from potato plants infested with mosaic and from mosaic tomato plants. Healthy, vigorously-growing tomato plants were inoculated, some with potato mosaic virus, some with tomato mosaic, and others again with a mixture of these two juices. The complete series which has been tested twice in the field and twice in the greenhouse is summarized in table 6.

The symptoms obtained when tomato mosaic and potato mosaic viruses were used were identical with those of tomato streak produced under similar conditions. Such symptoms did not develop in plants inoculated with tomato mosaic alone or potato mosaic alone. This streak derived from the combination virus could thereafter be transmitted to tomato plants.

These results at once opened up a new field of study. Since tomato and tobacco mosaic were known to be interchangeable, the latter was mixed with potato mosaic and the combination virus inoculated into tomato, with similar results as shown in table 6, exp. 3.

It also suggested the possibility of obtaining streak by inoculating mosaic tomato plants with potato mosaic, or, conversely, inoculating tomato mosaic into tomato plants which had three weeks previously been inoculated with potato mosaic. Accordingly, one of the plants of inoculation exp. 4, and one of exp. 5, table 6, were inoculated with potato mosaic three weeks after their first inoculation. At this time both plants showed definite mosaic symptoms only. Similarly, three weeks after the first inoculation, one of the plants of table 6, exp. 6, was inoculated with tomato mosaic. In all of these cases streak symptoms invariably developed 10–15 days after the second inoculation (table 6, exp. 8 and 9).

Further evidence that streak was caused by the combined viruses of tomato and potato mosaic was adduced when streak was obtained in mosaic tomato plants on which aphids from mosaic potato plants had been placed two to three weeks previously.

TABLE 6.—*Effect of inoculating healthy tomato plants with a combination of potato and tomato viruses*

Experiment No.	No. of tomato plants inoculated	Inoculum	Results 11–15 days after inoculation[a]
1	2	Tomato streak	Mosaic + streak
2	4	Tomato mosaic + potato mosaic	Mosaic + streak
3	4	Tobacco mosaic + potato mosaic	Mosaic + streak
4	2		Mosaic
5	2	Tobacco mosaic	Mosaic
6	2	Potato mosaic	No positive results
7	2	Control plants	Healthy throughout
8	2 mosaic plants	Potato mosaic	Mosaic + streak
9	2 plants inoculated 3 weeks previously with potato mosaic	Tomato or tobacco mosaic	Mosaic + streak

[a] Observations 4 weeks after inoculation showed no change.

Dried tomato streak material combined with potato mosaic. When streak tomato tissue, which was highly infectious when fresh, was dried for several months, then macerated and inoculated into tomato plants, mosaic only was produced. Potato mosaic virus was mixed with some of the macerated material and then inoculated into healthy tomato plants. This experiment was conducted both in the field and in the greenhouse with the results shown in table 7.

The symptoms which developed in all of the plants in table 7, exp. 2, were apparently identical with those produced on tomato plants with the fresh streak tomato material several months previously. From the above results it would appear, therefore, that the potato mosaic virus is the "factor" in the streak tomato material which was destroyed by drying.

Potato streak combined with tomato mosaic. In some recent preliminary experiments to ascertain if any relationship existed between potato

TABLE 7.—*Results of inoculating healthy tomato plants with macerated dried streak tomato tissue combined with potato mosaic*

Experiment Number	Number of tomato plants inoculated	Inoculum	Results
1	4	Macerated dried streak tomato tissue	Severe mosaic only produced
2	4	Do + potato mosaic	Mosaic + spots on leaves and streaks on stem (streak)
3	2	Potato mosaic	A peculiar mottling, distinct from tomato mosaic, was evident in some cases

and tomato streak, 8 tomato plants were inoculated with potato streak (from Spaulding Rose variety obtained from the Province of New Brunswick) and 8 with the combined viruses of potato streak and tomato mosaic. In the former case 5 of the plants produced a peculiar mottling distinct from true tomato mosaic, and 3 showed no apparent changes, whereas in the latter case, streak developed in all the plants. All controls remained healthy. Although only a few preliminary experiments have been attempted, the writer has been unable to produce streak in tomato from potato streak alone. The relationship between streak of potato and streak of tomato needs further elucidation.

A single series of inoculations have revealed the fact that the combined viruses of tomato and potato mosaic may be passed through tobacco and pepper, in both of which mosaic only develops, and yet produce streak on tomato. In other words, tobacco and pepper can act as intermediate hosts. Similar experiments with other Solanaceous plants are now in progress.

DISCUSSION

The results of the experiments embodied in this paper lead to the conclusion that streak disease of tomato at Macdonald College belongs to the "filterable virus" group of plant diseases.

The results show plainly that the disease is of a systemic nature, since leaves and fruit, themselves not visibly affected but coming from otherwise diseased plants, can be carriers of the inoculum. The presence of bacteria in the lesions could never be demonstrated definitely in stained sections. That bacteria are frequently found associated with mosaic tissue has been observed by other writers (14). A yellow bacterial organism, which is apparently identical with *Bacillus lathyri*, has repeatedly been isolated from streak lesions, but no further evidence has been obtained to show that it is other than secondary.

The fact that streak juice is still infectious after being passed through a bacteria-proof Chamberland F filter; after being subjected to a temperature of 70° C. for ten minutes; and finally, after being treated with strengths of mercuric chloride injurious to bacteria, for one to two days, definitely demonstrates that streak disease of tomato in Quebec is not of bacterial origin.

That the disease is not solely a physiological or malnutritional one is shown by its infectious nature. Even if tomato plants are grown under conditions which are recommended as preventive measures, and then inoculated with streak juice, streak will develop in nearly 100 per cent of these plants, although only minor symptoms will be evident in some cases. To date, all attempts on the part of the writer to produce the disease in healthy plants by manipulating the environment have failed. In cases where others have produced the disease at will, it is possible that the infective agent was latent in those plants only to become potent when conditions were favorable for its development.

All previous experimental work indicated that tomato mosaic was one of the chief, though apparently not the only, contributing cause of streak. On this continent the two diseases were invariably mentioned as being present in the same plant. The results of the experiments with the combination viruses reported herein very strongly indicate that potato mosaic is the other contributing factor. More work is necessary to determine whether other mosaic diseases may function as potato mosaic.

SUMMARY

The experimental studies reported herein, under both field and greenhouse conditions, show that:

(1) Bacteria, which are found associated with diseased lesions, are not the cause of tomato streak.

(2) The disease is highly infectious and can be transmitted from plant to plant by rubbing the leaves of healthy plants with the macerated tissue of diseased plants.

(3) The disease is of a systemic nature, since leaves not visibly affected are infectious.

(4) Streak tomato juice is still infectious after passage through a bacteria-proof filter, can usually withstand a temperature of 70° C. for 10 minutes without losing its infectivity, and can withstand concentration of mercuric chloride which readily destroys bacteria and fungous spores.

(5) Air-dried material soon becomes non-infectious.

(6) Fertilizer treatments alone cannot prevent the disease under all greenhouse conditions.

(7) Streak in Quebec has not been transmitted through the seed.

(8) Transmission by aphids has been proved.

(9) Failure has attended all attempts to induce the disease by manipulation of environmental factors.

(10) Excessive humidity, high temperature, and succulent growth are predisposing factors. Therefore control measures should consist of guarding against all predisposing factors, keeping the greenhouse free from all volunteer Solanaceous plants, and treating regularly for insect carriers.

(11) Tobacco may serve as a carrier of streak.

(12) A combination of potato mosaic and tomato mosaic viruses, when inoculated into tomato, produces symptoms identical with those of true streak, and all evidence indicates that these combined viruses are the true cause of the trouble.

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ANTHRACNOSE OF PEPPER (CAPSICUM ANNUUM L.)[1]

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With Plates XXII and XXIII

The first published report, which I have been able to find, of an anthracnose disease of pepper was made by Halsted (2) in 1890. The disease was attributed to *Gloeosporium piperatum* E. & E. In the same report another anthracnose of pepper is mentioned and attributed to *Colletotrichum nigrum* E. & H. A somewhat fuller description of this latter species is given in another article (3) published during the same year. Later, as a result of cross inoculation work with anthracnose fungi from several host species, Halsted (5) apparently became doubtful as to the real identity of the pepper *Gloeosporium*.

In 1898 Miss Stoneman (12) obtained the perithecial stage in single spore cultures of what she supposed to be *Gloeosporium piperatum* E. & E. and named the fungus *Gnomoniopsis piperata* n. gen. and n. sp.

Later H. von Schrenk and Spaulding (10) changed the generic name to *Glomerella* because the name *Gnomoniopsis* had been used by Berlese for an entirely different group of Ascomycetes six years previous to Miss Stoneman's publication.

Shear and Wood (11), after studying the perithecial and conidial stages of anthracnose fungi from a large number of host plants, drew the conclusion that those from the majority of the hosts (including pepper) were identical and should be referred to *Glomerella cingulata* (Stoneman) von S. and S.

During recent years several severe outbreaks of pepper anthracnose have been attributed to *Colletotrichum nigrum:* but, so far as I can learn, no experimental evidence as to the relation of this organism to the decay has ever been published.

Dastur (1), in reporting a study of pepper anthracnose in India and Burma, says of *Gloeosporium piperatum* E. & E. and *Colletotrichum nigrum* E. & Hals.: "These fungi are considered to be identical and to be the conidial forms of *Glomerella cingulata* (Stoneman) Spald. and von Sch."

The present study was begun during the fall of 1919 for the purpose of determining the mode of infection and the means by which the organism or organisms live over winter. Observations indicated that, in fields where pepper had been grown for two or three years and diseased material allowed

[1] Paper number 19, Journal Series, Georgia Agr. Exp. Station.

to rot in the field, the disease was little, if any, worse than in fields where pepper had never been grown before. Preliminary inoculation tests indicated that at least some forms of *Gloeosporium* occurring on pepper fruits were not actively parasitic. They were not capable of infecting sound fruits.

Practically all inoculation tests previously reported were made on wounded fruits. Dastur (1) stated that the fungus was able to enter through the unbroken cuticle, but few details of these inoculation tests were given and one could not be certain that the inoculated fruits were free from cracks or small punctures. Following our failure to produce infection on sound fruits, these cracks came under suspicion as the points of attack by the fungus. Selection for the purpose of developing a type of Pimento less subject to cuticle cracks was actually begun in 1920, but was abandoned when later results showed these cracks to be of little or no consequence in the development of fruit rots.

ISOLATIONS AND INOCULATION EXPERIMENTS

The first isolation of *Gloeosporium* was made during the summer of 1919 from a partly decayed ripe Pimento pepper fruit. A great many inoculations were made on Pimento fruits of all ages with both spores and mycelium of the fungus, but only a few infections resulted on wounded fruits and none on those not wounded. In all cases where infection occurred, the decay did not spread to any appreciable extent until the host fruit began to ripen.

During the following spring some Chili pepper growing in the greenhouse became badly diseased. Comparatively small spots, usually one to two centimeters in diameter, appeared on the young green fruits as well as on the ripe fruits. The spots were sunken, circular to oblong in outline, only slightly changed in color, and bearing large orange-yellow acervuli usually arranged concentrically on the spot. The conidia were slightly larger and more uniform in size than those isolated the previous year. Both the spots and the associated organism seemed to correspond in every way with the description of *Gloeosporium piperatum* E. & E. as given by Halsted. Elongated, grayish spots were also produced on the branches and main stem of the plants, these in some cases girdling and killing the entire plant.

Spores were scraped from one of the fruit spots, suspended in water, and applied with a sterile camel's hair brush to 5 green pods of Chili, 5 Pimento, and 6 Bell peppers. The inoculated pods, with a like number of checks, were wrapped with moist absorbent cotton and kept moistened for 5 days. At the end of 7 days the cotton was removed. At this time spots were evident on some of the inoculated pods, and, by the tenth day,

typical anthracnose spots with orange yellow acervuli were found on four Chili, one Bell, and two Pimento pods. One of the Pimento pods had 30 spots and the other about a hundred. None of the checks were diseased.

A single spore culture was obtained from a diseased Chili fruit and was later used in making a large number of inoculations. Infections were produced readily on young fruits less than two inches long, but less frequently on older fruits after the cuticle had begun to thicken.

During the fall of 1921 a *Gloeosporium* was isolated from a pale green spot on the cotyledon of a young seedling from commercial pepper seed. The morphological and cultural characters were similar to those of the form from Chili fruits, and successful inoculation of young pepper plants by spraying with a spore suspension and of young unwounded fruits by the previously described method proved it to be actively parasitic.

During the summer of 1924 an anthracnose, produced by what appeared to be the same species of *Gloeosporium*, was very severe on a small plat of Bell pepper in the field. More than 50 per cent of the fruits were destroyed. During periods of moist weather it was difficult to find a sound ripe fruit. Single spore cultures were obtained from a diseased fruit; and conidia from these cultures were used in inoculating approximately 100 fruits of all ages. The spores were suspended in water and applied by various methods over the surface of the unwounded fruits, which were then wrapped with moist absorbent cotton and kept moist as in previous experiments. At the same time a similar number of uninoculated fruits were wrapped and kept moist as checks. About 75 per cent of the inoculations were successful. Those inoculated before the cuticle of the fruit had thickened, or after they had turned red, gave almost 100 per cent infection, but the nearly mature green fruits were resistant and rarely infected. This is doubtless due to the mechanical resistance of the thick cuticle against the entrance of the germ tube. None of the checks became infected.

About the same time, a *Glomerella* (apparently *G. piperata* (Stoneman) von Sch. & S.) was isolated (single ascospore cultures) from a pepper fruit from another field. Both conidia and ascospores from these cultures were used in inoculating 50 fruits by the same method used in the above described experiments, except that a few of the fruits were wounded. Three of the wounded ripe fruits developed spots; but none of the wounded green fruits and no unwounded fruit, either ripe or green, showed any evidence of infection.

These inoculations with *Glomerella* were made on greenhouse grown plants from the same lot, at the same time, and under identical conditions as some of the inoculations with the parasitic form from Bell pepper. The *Glomerella* is evidently only very weakly parasitic, a wound parasite of ripe fruits.

Gloeosporium was again found in a field of Paprica pepper during the summer of 1925. The fungus was isolated and proved to be actively parasitic by its ability to infect sound fruits. Twenty-one Bell pepper fruits of various ages were inoculated without wounding, by methods previously described, with conidia from a single spore culture. Eight, or 38 per cent, developed typical anthracnose spots. At the same time 26 similar fruits were inoculated with conidia from a freshly isolated culture of *Glomerella piperata*. None of these showed any evidence of infection.

Further positive evidence of the parasitism of the form from Chili pepper fruits was gained from a histological study of inoculated spots.

HISTOLOGICAL STUDY OF THE DISEASE

Conidia from a culture of the actively parasitic *Gloeosporium* from Chili pepper were suspended in sterile water, to which a little powdered chalk had been added as a marker. With a sterile pipette droplets of this spore suspension were placed on the surface of young pods of Chili pepper. The pods were then wrapped with moist absorbent cotton. At the end of two, three, five, and seven days some of the inoculated spots were cut out and killed in Gilson's mercuric chloride solution. The material thus obtained was embedded in paraffin, sectioned, and stained with various stains.

At the end of 48 hours the spores had germinated and had formed appresoria which clung to the host cuticle, but no cases of actual infection were found. In material killed the third day many infection tubes from the appresoria had passed through the cuticle and had begun to kill the surrounding cells (Pl. XXIII, Figs. 13–15). By the fifth day the fungous mycelium had spread extensively through the host tissue, large areas of the host tissue were killed, and in one case an acervulus was beginning to form (Pl. XXIII, Fig. 16). The material killed the seventh day was not well fixed and could not be used.

For a more general study of the acervuli and of the more advanced stages of decay of the host tissue, naturally infected spots in various stages of development were killed, sectioned, and stained by similar methods.

In many cases the host cuticle directly beneath the appresorium was changed in some way so that it was stained an intense black by Haidenhain's haematoxylin when other portions of the cuticle remained almost colorless. Apparently this occurred only when the appresorium was beginning to germinate. Usually, in such cases, one or more of the epidermal cells directly beneath the appresorium were dead, as indicated by the coagulation of the protoplasm and disintegration of the cell nucleus (Pl. XXIII, Fig. 13). Appearances indicate that the germinating appresoria secrete an enzyme, or other substance, which dissolves, or at least softens, the cuticle, and also some toxic substance which penetrates and kills the neighboring host cells.

As the appresorium germinates, a slender infection tube from the under surface passes directly through the cuticle, passing sometimes directly into an epidermal cell and sometimes between cells. As infection proceeds, the host cells are killed a distance of two to three, and sometimes more, cells ahead of the advancing hyphae. The middle lamella very quickly disappears, and the cell wall of the host is split into very thin lamellae (see cells in Pl. XXIII, Figs. 16 and 19), the number depending upon the thickness of the cell wall. In the thick-walled cells near the epidermis, as many as 8 lamellae could be counted in the partly decayed cell wall, while in the large thin-walled cells of the interior there were usually only two.

Penetration of the cell walls by the fungous hyphae appears to be effected partly by the dissolving action of an enzyme and partly by mechanical force. In early stages of decay no indications of mechanical force are found; but in later stages the elements of the cell wall are usually pushed out of place (Pl. XXIII, Fig. 17), indicating that considerable force is exerted by the advancing hyphae. Cutinized and lignified membranes are attacked very slowly. Hyphae enter the lignified cell walls of the vascular bundles through very small openings (Pl. XXIII, Fig. 18), apparently always through pits. The middle lamella soon disappears, but the lignified portions remain apparently intact when the surrounding tissues have entirely distintegrated. Corrosion of the cutin is quite evident where hyphae are in direct contact with it, but this action is only local and very slow. In mature pepper fruits the cell walls are cutinized to a depth of 3 to 8 cell layers, the cutin being deposited between the cells. In old anthracnose spots on such mature fruits, the cellulose walls are disintegrated and split into their elemental lamellae while held together by the apparently unchanged cutin deposit (Pl. XXIII, Fig. 19).

The greatest vegetative growth of the fungus is within the inner soft tissue with thin cell walls; but, when acervuli begin to develop, the epidermal cells become packed with mycelium. As the conidiophores develop, the outer wall of the epidermal cell is pushed up with the cuticle. Sometimes conidiophore layers are again formed in the underlying cells, and the first layer is pushed up to become a part of the loose covering of the new acervulus.

The mycelium also pushes into the internal cavity of the fruit and attacks the placentae and seed, often killing the embryo. Whether or not the mycelium remains dormant in the coat of viable seed has not been determined; but it has been isolated from cotyledons of seedlings from seed of a diseased Chili pod that had been soaked two minutes in a 1–1000 solution of mercuric chloride.

OTHER ANTHRACNOSE ORGANISMS ISOLATED FROM PEPPER

In testing various lots of commercial seed, a large percentage of the seedlings from some lots developed anthracnose spots on the cotyledons or hypocotyls, when grown under moist conditions. On some the acervuli had abundant setae, while others showed no setae whatever. Isolations were made from a number of these that appeared to be different.

Our experience with the first organism isolated, indicating that it was not actively parasitic, together with observation in the field, led to the suggestion that perhaps we were dealing with several closely related organisms. During the summer and fall of 1921 a systematic search was made in the pepper fields for pepper pods bearing anthracnose spots of peculiar appearance. Notes were made as to the general appearance of the spots and as to the microscopic characters of the causal organism. Single spore cultures of the organism present were isolated from each spot thus noted. The cultures were numbered and labeled *Colletotrichum* or *Gloeosporium* in accordance with the presence or absence of setae in the acervuli of the original spot.

Including those obtained from seedlings, 19 cultures were isolated and kept growing on culture media until intensive study and comparisons could be made. This was not possible until the summer of 1923, when the cultures had been on artificial media one and a half to three years and two had been lost.

The 17 remaining cultures were grown on cornmeal agar, cornmeal mush, oatmeal agar, glucose agar, steamed bean pods, steamed pepper stems, and steamed tomato stems. Further isolations were made during the course of this study in 1923 and these were used also in some of the comparisons.[2]

The glucose agar had an initial reaction of pH 7.2. An indicator, brom thymol blue, was added before sterilization. Glucose broth was also prepared in the same manner. On both media the H-ion concentration at first increased to the yellow color of this indicator and then decreased to the full blue color of the indicator. There was considerable variation in the time required for these changes in reaction; but the general course of the change was the same in all cultures, indicating that the reaction would probably have little value in separating species in this group of fungi.

On the other media the color, luxuriance, and general appearance of the mycelial growth, the presence or absence of acervuli, their color when present, the size and shape of conidia, the presence or absence of setae, the presence of sclerotia, and the presence or absence of perithecia were noted and tabulated for easy comparison.

[2] Most of the comparisons were made by Dr. Adeline Ames while working as a special assistant during the summers of 1923 and 1924. Thanks are due to her for careful notes on the cultures and also for some of the drawings in Plate XXII.

In general the cultural characters were not sufficiently distinct and constant to be of much descriptive or diagnostic value. Some of the more distinct forms did have distinctive characteristics in culture, but these were less striking than the morphological and other characters on the host plant. For instance *Colletotrichum* No. 3 and No. 5 produced in young cultures on cornmeal a burgundy red color in the substratum, but this color gradually disappeared and was not distinctive in older cultures.

The presence or absence of setae was not constant in a few cultures. In three originally labeled *Colletotrichum*, no setae could be found in some cultures and two labeled *Gloeosporium* occasionally produced what appeared to be setae. It seems doubtful that they should be classed as true setae in either case. The spots from which some of the *Coletotrichum* cultures were obtained showed dark seta-like structures in older parts of the spots. These were generally rather elongate, much septate, thin-walled, and with a general appearance of being abortive conidiophores. Cultures from forms of a distinctly *Colletotrichum* type such as Nos. 3 and 5 (described under *Colletotrichum* sp.) always produce abundant setae. Even when conidia were found on scattered conidiophores, they were usually accompanied by one or more setae (Pl. XXII, Figs. 8 and 9).

Another, which appears to be identical with *Colletotrichum nigrum*, was isolated three different times; but in all cultures few or no conidia were produced and usually no setae. On all media tried, the cultures turned black after a few days, with innumerable small black sclerotia. Young cultures from spores usually produced conidia; but sub-cultures made by transfer of mycelium usually produced only sclerotia.

In all the cultures, the conidia produced on artificial media were slightly more variable in size than those produced on pepper fruits, but always of the same shape and general type. For instance, the conidia of *Colletotrichum* Nos. 3 and 5 were curved and had pointed ends on all media.

Two of the original cultures (one of *Colletotrichum* and one *Glomerella* from ascospore) and two of *Gloeosporium* isolated during the summer of 1923 produced perithecia in culture. Perithecia were also found quite commonly on diseased pods in the field during 1923. Upon the characters of perithecia and ascospores, however, these could be separated into two distinct groups, apparently distinct species.

IDENTITY OF ORGANISMS STUDIED

The characters of five of the forms studied were so distinct as to leave little doubt of their being separate species. The actively parasitic *Gloeosporium* isolated from spots on Chili pepper fruits in 1920, from cotyledon of seedling in 1921, from Bell pepper fruit in 1924, and from Paprica in 1925, are all apparently identical with *Gloeosporium piperatum* E. & E.

The description of this species is emended as follows:

Gloeosporium piperatum E. & E. Spots circular to elliptical in outline, 1–2 centimeters in diameter, depressed, dirty green to yellowish, turning brown in age. Acervuli formed within the epidermal or subepidermal cells, erumpent, circular to oblong, yellowish orange to salmon color; Conidia one celled, short rod-shaped, 5.4 – 6.2 μ by 15.5 – 18.64 μ, ends rounded, hyaline, with prominent nucleus showing as refractive spot in center.

Parasitic on fruits, stems, and leaves of *Capsicum annuum*.

Colletotrichum nigrum E. & H. Spots irregular in outline, indefinite, depressed, blackish; acervuli numerous, scattered over the spot, sub-cuticular, sub-epidermal or superficial, up to 100 μ in diameter, black with numerous setae; setae dark brown, septate, stout, tapering upward, 2 – 6.2 μ by 75 – 100 μ; conidia, hyaline, cylindrical, 3.5 – 5.3 μ by 18.6 – 25 μ, ends blunt. Small black sclerotia found within the host tisue and in culture media.

Wound parasite on fruits of *Capsicum annuum*.

Colletotrichum nigrum shows evident close relationship with *C. tabafica* (Hallier pro-parte) Pethybridge (9) and other sclerotium-producing species.

Colletotrichum sp. Spots irregular in outline, indefinite, depressed, dark brown to blackish; acervuli numerous, black; setae numerous, septate, stout, straight or slightly bent, tapering upward, dark brown to black; conidia one celled, hyaline, sickle-shaped, ends pointed, 4 – 5.4 μ by 18.6 – 25 μ. Small black sclerotia formed in cultures and occasionally within the host tissue.

Wound parasite on fruits of *Capsicum annuum*.

This species is possibly identical with *Colletotrichum omnivorum* Halst. (4) but apparently differs from it in size of spores and greater abundance of setae.

Glomerella piperata (Stoneman) von Schrenk & Spaulding. Perithecia scattered, partly embedded in the host tissue or in a more or less stromatic mass of hyphae, globose to oval or pear-shaped, smooth or strigose with dark green hyphae about base and occasionally about ostiole, ostiolate, sometimes slightly beaked; asci linear-club shaped; ascospores hyaline with a prominent central nucleus, linear, curved, 4 – 6 μ by 18 – 24 μ, germination usually direct without formation of cross wall.

Conidia. Spots pale straw color with dark acervuli scattered over older parts; acervuli erumpent, small, setose and dark in age; setae black, septate, tapering, few to numerous; conidia continuous, hyaline, cylindrical, 3.5 – 6 μ by 10.5 – 25 μ.

This species is probably identical with *Gnomoniopsis piperata* Stoneman, but positive identification seems well nigh impossible at the present time, since the only type material (prepared slides) of *G. piperata* now available show few of the characters of the fungus. The perithecia were described by Miss Stoneman as usually long beaked, whereas ours have rarely shown a well developed beak; but the asci and ascospores agree so closely with her description that there is little doubt in my mind as to its identity.

Glomerella sp. Perithecia scattered or in small groups, embedded in the host tissue, finally erumpent or in a more or less stromatic mass of hyphae, globose to flask shaped, usually long beaked; asci slender club-shaped, 12 by 40 – 80 μ; ascospores hyaline to smoky, straight, oblong, 6 – 9 μ by 12 – 18 μ, at first continuous but uniseptate in old perithecia and always becoming septate before germination.

Conidial stage: spot circular or often irregular in outline, indefinite, dark colored towards center; acervuli scattered, erumpent, at first pale yellow or straw colored, sometimes slightly setose in age; conidia hyaline, short cylindrical, 4 – 6.5 μ by 16 – 21 μ, continuous, becoming uniseptate before germination.

Wound parasite on fruits of *Capsicum annuum*.

The fact that several species of anthracnose-producing organisms occur on pepper pods offers a probable explanation of the very divergent opinions expressed as to the identity of the pepper anthracnose organism and its relation to anthracnose on other plants. Apparently *Gloeosporium piperatum* is not nearly so common on pepper pods as are some of the other weakly parasitic forms. The latter infect blosson-end rot spots and other wounds and are nearly always present in pepper fields. They are, therefore, the forms most frequently studied and reported as pepper anthracnose.

Often two or more anthracnose organisms and other saprophytic fungi occur on a single spot. In one case, while isolating *Colletotrichum nigrum* from an original blossom-end rot spot, one single spore culture proved to be *Glomerella piperata*. Fortunately the fruit from which the isolation had been made was saved. Upon more careful examination it was found that the two organisms could be distinguished as distinct colonies which had intermixed along the edges, and Glomerella pirithecia were present on portions of the spot.

No such mixture of organisms has been found in spots produced by the parasitic form here reported, although this also seems entirely possible.

From my own observations, I am led to doubt Miss Stoneman's having obtained the ascigerous stage of *Gloeosporium piperatum*. I have never found any indication of perithecia either in cultures of what I have here

considered *Gloeosporium piperatum* or on pepper fruits affected with this form.

Several workers have made comparisons of anthracnose organisms isolated from various hosts and upon these comparisons have based the conclusion that they all belonged to the same species. Cross inoculations have, in most cases, been made by placing the fungus in wounds on more or less fleshy fruits. Under the conditions of these experiments any such weakly parasitic, poorly specialized organism might attack almost any fleshy fruit. Such cross inoculation experiments are without serious value in determining species in such an omnivorous group of fungi.

Furthermore, any of these wound parasites may be found on wounded fruits or plant parts of other species of plants, which may account for the widespread occurrence of what Shear and Wood (11) and others have considered *Glomerella cingulata* isolated from various hosts.

The opinion expressed by Dastur that *Gloeosporium piperatum* and *Colletotrichum nigrum* E. & H. are one and the same fungus and that both are identical with *Glomerella cingulata* does not appear to be well founded. From his discussion I am led to doubt his having worked with *Gloeosporium piperatum*, and feel certain that he never worked with *Colletotrichum nigrum*. These two have so few characters in common that I do not believe that anyone, having compared them, could say that they are identical.

OVERWINTERING OF GLOEOSPORIUM PIPERATUM

With many parasitic Ascomycetes the perithecial stage matures in early spring and the ascospores serve as the principal, and often the only, source of spring infection; but, in the section of *Gloeosporium* to which *G. piperatum* is most closely related, the perithecial stage develops along with the conidia and apparently does not play any very important role in the propogation of the fungus. Possibly the ascospores are more resistant than conidia to unfavorable environmental conditions and may be able to live in the soil for a longer period. But, if *G. piperatum* has a perithecial stage, it develops so rarely as to be unimportant in disseminating the fungus.

The longevity of the conidia in soil or under field conditions has not been determined; but there is no indication that anthracnose is worse in fields where pepper has been grown for three or more consecutive years than in fields where pepper has never been grown before. Halsted (6) reported that application of decayed fruits and stems from previous crops did not increase the amount of disease. From observation I am led to believe that seed-borne spores are the most important source of primary infection.

The results of seed treatment tests and examination of seedlings previously reported (7) indicate that the spores of *Gloeosporium piperatum*, as

well as those of other fungi, become attached to the surface of the seed during the process of washing and drying and infect the young seedlings as they emerge from the seed coat. The fungus persists in rather inconspicuous spots on the leaves and stems of the plants until fruits are formed. This is probably the most important source of pod infection.

The amount of seedling infection can be greatly reduced by surface disinfection of the seed. Soaking 5 minutes in a 1–1000 mercuric chloride solution or 10 minutes in a 1–60 solution of copper sulphate followed by dusting with air slaked lime has been found effective for this purpose. Presoaking in water before either treatment increases the efficiency of the disinfectant.

SUMMARY

1. A study of pepper anthracnose has shown that several species of *Gloeosporium* and *Colletotrichum* occur on the fruits and other parts of the pepper plant. At least five distinct species have been isolated during the course of the study.

2. Of these, only one species, apparently *Gloeosporium piperatum*, is actively parasitic. This species attacks the fruits in all stages of development and occasionally produces spots and girdling of stem and branches.

3. *Gloeosporium piperatum* is comparatively rare in pepper fields.

4. The other four species are wound parasites, which enter and sometimes enlarge blossom-end rot spots and other wounds on pepper pods. These are the forms most common in the field.

5. *Colletotrichum nigrum* is a wound parasite on pepper pods. It has very distinctive characters and is not easily confused with *Gloeosporium piperatum* or any of the other anthracnose organisms found on pepper during the present study.

6. The perithecia of two species of *Glomerella* have been found on pepper pods. Both have Gloeosporium-like conidial stages.

7. The germinating spores of *Gloeosporium piperatum* produce one or more appresoria, which, after becoming firmly attached to the cuticle of the pepper pod, send infection tubes through the cuticle and into the interior of the pod.

8. The entrance through the cuticle is gained apparently by a cutin dissolving substance produced by the appresorium or by the infection tube.

9. The fungous mycelium secretes some toxic substance which kills the host cells before they are actually penetrated by the advancing hyphae.

10. The cellulose and hemi-cellulose membranes of the host tissue are readily destroyed by the fungus; but cutinized and lignified membranes are very resistant to its action, being decayed only very slowly.

11. The spores of the fungus become attached to the surface of the pepper seed during the process of cleaning and washing and attack the young seedlings as they emerge from the seed coat.

12. Surface disinfection of the seed greatly reduces the amount of seedling infection.

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EXPERIMENT, GA.

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DESCRIPTION OF PLATES

PLATE XXII

Drawings to illustrate morphological characters of five anthracnose fungi isolated from pepper fruits. All figures except 1 and 4 drawn to same scale with Zeiss 6 ocular and D objective, with the aid of a camera lucida.

Fig. 1. Perithecia of *Glomerella* sp. developed on steamed tomato stems.
Fig. 2. Asci of same species of *Glomerella*.
2a. Ascospores.
2b. Germinating ascospores.
Fig. 3. Conidia of same species of *Glomerella*.
3a. Germinating conidia.
Fig. 4. Perithecia of *Glomerella piperata*.
Fig. 5. Asci of *G. piperata*.
5a. Ascospores of *G. piperata*.
5b. Three germinating ascospores.
Fig. 6. Conidia of *G. piperata*.
6a. Three germinating conidia.
Fig. 7. Acervulus of *Colletotrichum* sp. developed on cornmeal agar plate.
Fig. 8. Conidia developing on scattered conidiophores on same medium.
Fig. 9. Conidia from steamed tomato stem.
Fig. 10. Conidia of *Gloeosporium piperatum* from green pepper fruit.
10a. Four conidia germinating in water.
Fig. 11. Conidia and setae of *Colletotrichum nigrum* from pepper fruit.
Fig. 12. Three germinating conidia of *C. nigrum*.

PLATE XXIII

All figures drawn with aid of camera lucida, Figs. 13, 14, 15, and 18 with Zeiss No. 8 ocular and B. & L. 1.9 mm. (Fluorite) objective, Fig. 17 with No. 12 ocular and 1.9 mm. objective, and Figs. 16, 19, 20, and 21 with No. 8 ocular and B. & L. 4 mm. objective.

Figs. 13–15. Germinating conidia and appressoria of *Gleosporium piperatum* on the cuticle of a young fruit of Chili pepper 72 hours after inoculation. In 14 and 15 germ tubes have penetrated the cuticle.

Fig. 16. Section of Chili pepper fruit 5 days after inoculation, showing mycelium passing through pits from cell to cell in the cutinized area and the beginning of an acervulus within the epidermal cells. Note disintegration of host cell walls still held in place by cuticle.

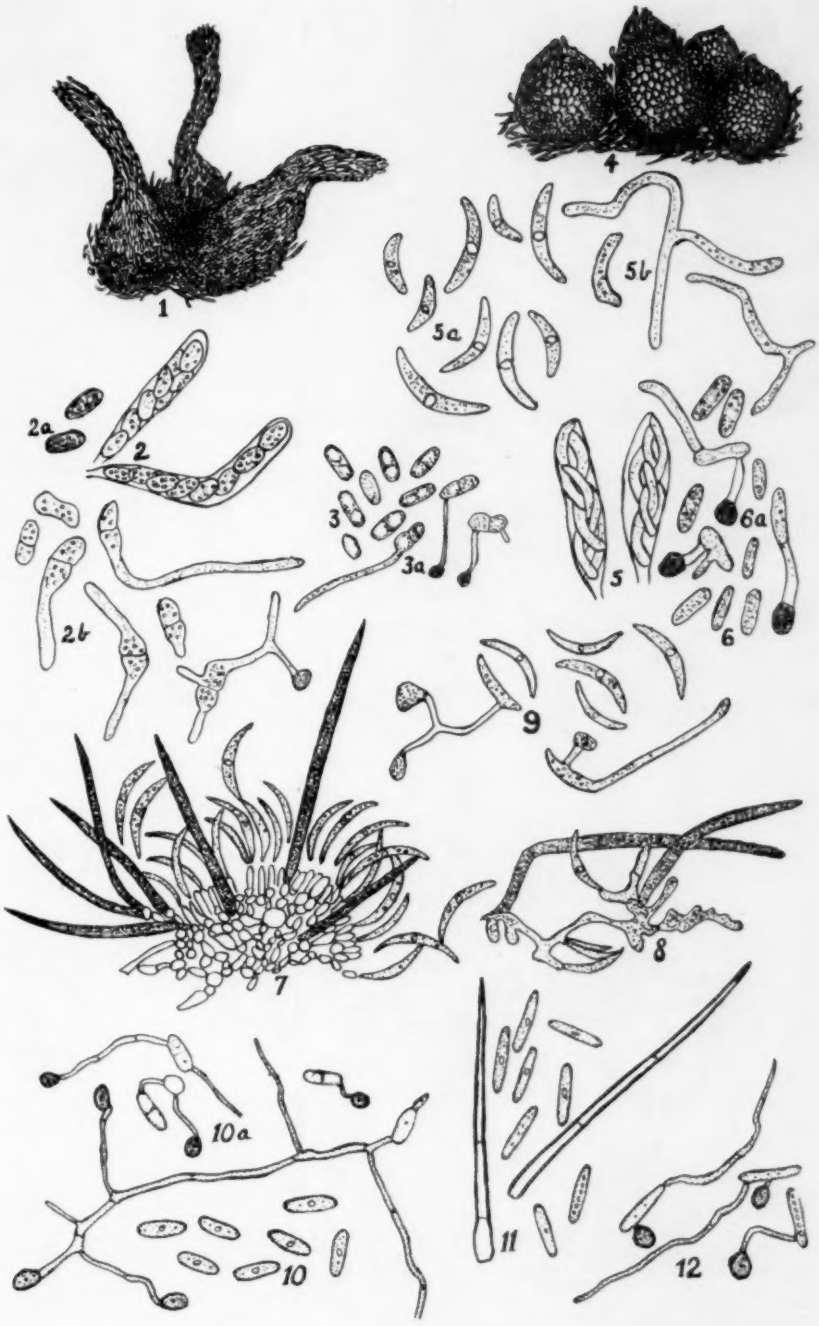
Fig. 17. Mycelium of *G. piperatum* passing through cell wall, showing dissolution of middle lamella and displacement of cellulose membrane by pressure from hypha.

Fig. 18. Mycelium in lignified ducts in a decayed area of Chili fruit.

Fig. 19. A young acervulus of *Gloeosporium piperatum* developing in the epidermal cells of a green Chili fruit, natural infection.

Fig. 20. Acervulus of *Colletotrichum nigrum* in decayed tissue of Pimento fruit.

Fig. 21. Section of sclerotium of *C. nigrum* within decayed tissue of Pimento fruit. The cells are mostly empty and dead soon after maturity.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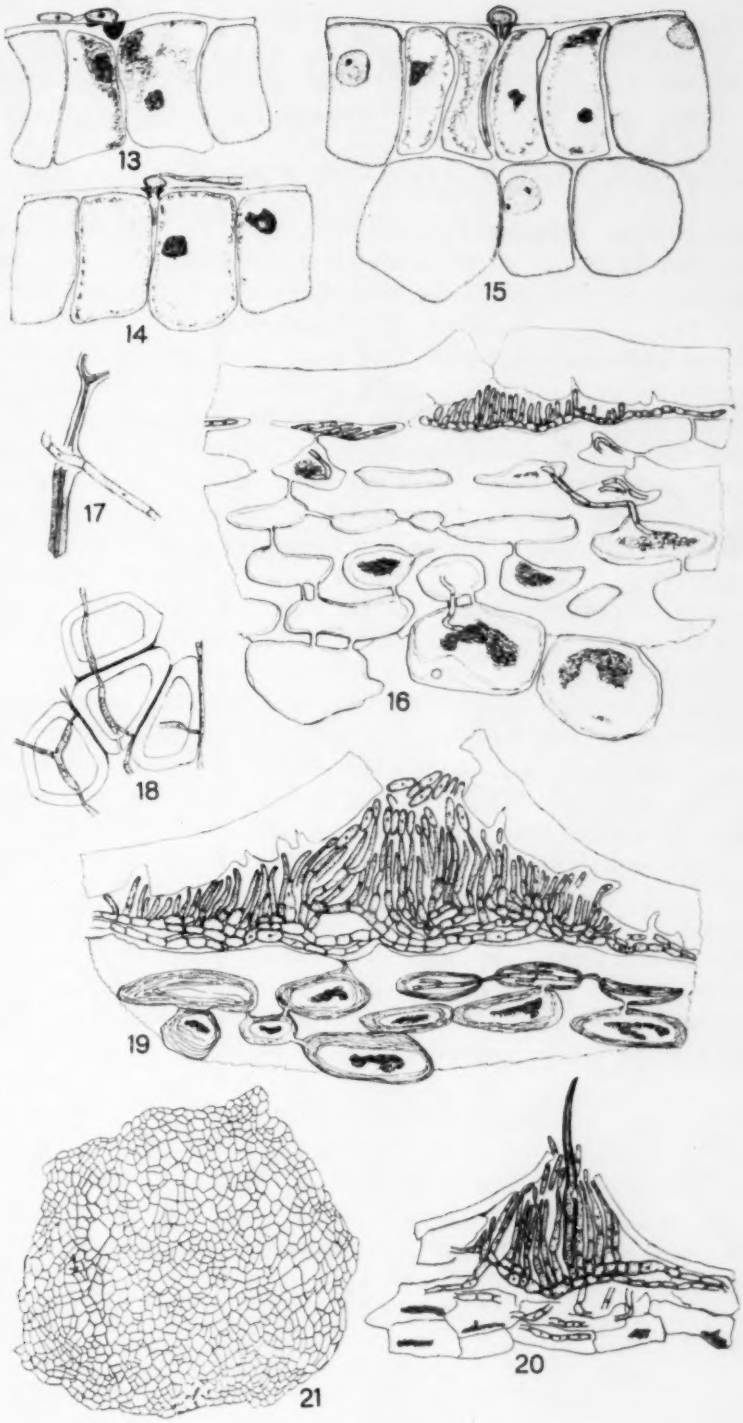
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UNIV. OF MICH.

FURTHER EXPERIMENTS ON THE CONTROL OF BUNT OF WHEAT AND THE SMUTS OF BARLEY AND OATS

R. W. LEUKEL

During the past few years much experimental work has been done on new seed treatments, both liquids and dusts, for the control of cereal smuts. Many new proprietary compounds have been developed for plant disease control, some of which have considerable merit.

From 1921 to 1924 about thirty such compounds were used in experiments at the Arlington Experiment Farm, Rosslyn, Va., in an attempt to find materials superior to bluestone and formaldehyde for the control of certain of the cereal smuts.[1] During the crop season of 1924–1925 the most promising of these compounds, together with others more recently received, were selected for further study. As the data from these studies, to a great extent, confirm those of previous investigations, it is thought desirable to publish them.

Experiments with treatments for the control of bunt were confined almost entirely to dusts, of which twenty were used. In order to test thoroughly the fungicidal properties of these dusts, Purplestraw wheat from the crop of 1924 was heavily dusted with bunt spores by mixing 1 part of bunt with 100 parts of wheat by weight. This mixture was shaken in a closed container so that the kernels were thoroughly coated with spores. The excess bunt was then removed by sifting. This procedure resulted in more heavily infested seed than probably is ever sown under ordinary field conditions. Bunt control by a compound under these circumstances, even if not perfect, should serve as an indication of its merits.

Inasmuch as the seed was very heavily bunted, the dusts also were applied quite heavily—at the rate of 8 ounces per bushel. Wheat usually will not retain more than 2 to 3 ounces per bushel, so, after the mixture had been thoroughly shaken in a closed container, the excess dust was removed by sifting. Three liquid treatments were used for purposes of comparison.

The treated seed was sown on Arlington Experiment Farm, Rosslyn, Va., October 13, 1924, at the rate of one gram per foot in 16-foot rows replicated several times. Germination tests were made by sowing 250 kernels

[1] Tisdale, W. H., Taylor, J. W., Leukel, R. W., and Griffiths, Marion A. New seed disinfectants for the control of bunt of wheat and the smuts of oats and barley. Phytopath. 15: 651–676. 1925.

from each lot of treated seed in 16-foot rows replicated four times, along with fifty similarly sown rows of untreated seed. Germination counts were made ten days after sowing. Germination tests also were made in greenhouse benches.

Infection counts were made in June, 1925. In those rows in which infection was light, all the bunted heads were counted. The average total number of heads per row was determined by counting the heads in a number of representative rows, and from this the percentages of infection were computed. In the more severely bunted rows the percentages of infection were arrived at by examining a hundred heads in each row.

The different rows were harvested and threshed separately, and the average yield in bushels per acre was computed for each treatment and for the corresponding untreated controls. The germination and infection data, together with the yields in bushels per acre, are shown in table 1.

Of the twenty dusts used, thirteen controlled bunt satisfactorily, taking the abundantly infested seed into consideration. Formaldehyde was the only treatment which completely eradicated bunt, but it reduced germination and yield considerably. Copper sulphate solution controlled bunt satisfactorily, but its effect on germination and yield also was undesirable. Five of the eight dusts furnished by the E. I. du Pont de Nemours and Company, Inc., were fairly satisfactory, but not all of these as yet are being manufactured in large quantities. Excellent results followed the use of Karasch Compounds "A" and "B," but these substances are not being made on a commercial scale. Bayer No. 3, Corona 640–S, and nickel carbonate also were fairly satisfactory. Copper carbonate, as in previous experiments, gave excellent results from the standpoint of germination, bunt control, and yield, even when it contained only 18 per cent of metallic copper. Among the commercially available dusts it seems unsurpassed for general use in bunt control.

Experiments were conducted on the control of covered smut of barley with a limited number of liquid treatments, some of which had given excellent results in previous experiments. Seed of Tennessee Winter barley, naturally infested and artificially inoculated with covered smut and contained in cheesecloth sacks, was immersed in the different solutions. After being allowed to dry, it was sown on Arlington Experiment Farm, October 10, 1924, at the rate of one gram per linear foot in 16-foot rows replicated twelve times. Germination counts were made in the field and in the greenhouse, as previously described for wheat. Infection counts were made May 14, 1925, by counting the infected heads in each row. The average number of heads per row was determined as in the case of wheat, and from this the percentage of smut was computed. These data are shown in table 2.

TABLE 1.—*Summary of data on seed germination in the field and greenhouse, bunt control, and yield in Purplestraw wheat from seed untreated or treated with different disinfectants and sown in 16-foot rows on Arlington Experiment Farm, Rosslyn, Va., October 13, 1924. Infection data taken June, 1925*

No.	Treatment	Germination		Replications	Bunt	Average yield
		Field	Greenhouse			
		(Pct.)	(Pct.)	(Number)	(Pct.)	(Bushels per A.)
1	Untreated control	62	71	13	46.5	17.4
2	Copper carbonate (C.P.)	65	78	13	0.84	27.5
3	Corona Coppercarb	71	85	13	0.65	28.5
4	Untreated control	66	70	13	54.4	17.6
5	Nichol's copper carbonate	71	84	13	0.48	28.4
6	Corona copper stearate	71	75	13	8.2	27.1
7	Untreated control	68	74	13	49.7	14.8
8	Semesan	70	84	13	0.74	25.8
9	Uspulun bolus	71	81	13	30.0	18.9
10	Untreated control	66	72	13	49.1	15.5
11	Bayer No. 3	73	88	13	1.16	26.7
12	Corona 640–S	71	77	13	1.2	26.9
13	Untreated control	67	73	13	51.5	16.8
14	Dupont No. 12	73	86	13	0.5	26.0
15	Dupont No. 13	73	83	13	26.3	25.0
16	Untreated control	68	68	13	49.5	17.7
17	Copper sulphate (dust)	63	69	13	3.0	23.8
18[a]	Copper sulphate (solution)	58	76	13	0.55	25.5
19	Untreated control	66	69	6	48.0	18.9
20	Flowers of sulphur	68	76	6	16.0	27.1
21[b]	Colloidal sulphur	70	77	6	32.0	23.9
22	Untreated control	65	67	6	38.8	19.6
23	Karasch Compound A	70	84	6	0.86	29.2
24	Karasch Compound B	70	81	6	0.1	30.7
25	Untreated control	68	62	6	44.6	19.9
26	Nickel carbonate	69	80	6	2.6	28.4
27	Dupont No. 16	70	75	6	2.4	27.6
28	Untreated control	66	64	6	48.3	19.6
29	Dupont No. 18	76	83	6	0.5	28.1
30	Dupont No. 19–B	78	83	6	22.5	23.3
31	Untreated control	67	61	6	45.1	18.8
32	Dupont No. 9	75	88	6	0.2	30.5
33	Dupont No. 30	74	80	6	28.8	25.4
34	Untreated control	67	65	4	46.5	16.6
35[c]	Formaldehyde	40	50	10	0	19.0
36	Untreated control	64	67	3	44.5	19.5

[a] 1–10 dip.

[b] 1 per cent solution, 1 hour.

[c] 1:320 for 10 minutes.

TABLE 2.—*Summary of data on control of covered smut in Tennessee Winter barley and of covered and loose smuts in Aurora oat from smutted seed, untreated, or treated with various disinfectants, and sown on Arlington Experiment Farm, Rosslyn, Va., October 10, 1924, in single 16-foot rows, replicated twelve times for barley and fourteen times for oats. Infection data taken in May, 1925*

No.	Treatment	Strength	Time	Germination		Smut
				Field	Greenhouse	
		(Pct.)	(Hrs.)	(Pct.)	(Pct.)	(Pct.)
			Barley			
1	Control	–	–	78.	90.	8.
2	Uspulun	.3	1	79.	96.	0.04
3	Germisan	.3	1	85.	93.	0.
4	Control	–	–	84.	95.	8.
5	Semesan	.3	1	84.	94.	0.3
6	Corona 640–S	.3	1	84.	91.	9.
7	Control	–	–	82.	96.	7.
8	Formalin	.3	1/2	64.	80.	0.06
9	$CuSO_4$	2.4	1/6	84.	92.	1.
10	Control	–	–	81.	94.	8.
11	Colloidal Sulphur	.5	1	78.	96.	7.
12	Water	–	1	84.	95.	15.
			Oats			
1	Control	–	–	85.	92.	8.
2	Uspulun	.3	1	76.	94.	0.
3	Germisan	.3	1	75.	96.	0.
4	Control	–	–	82.	96.	9.
5	Semesan	.3	1	79.	95.	0.07
6	Corona 640–S	.3	1	78.	96.	9.
7	Control	–	–	82.	94.	10.
8	Formalin	.3	1/2	68.	95.	0.
9	$CuSO_4$	2.4	1/10	79.	96.	0.3
10	Control	–	–	81.	97.	9.
11	Colloidal Sulphur	.5	1	76.	98.	0.7
12	Water	–	1	71.	98.	12.

Germisan at .3 per cent for one hour eliminated covered smut completely, while Uspulun and Semesan reduced it to a very slight trace. Formaldehyde also gave fair control but reduced the percentage of germination. Corona 640–S, copper sulphate, and colloidal sulphur were unsatisfactory. Soaking the seed in tap water for one hour seemed to increase greatly the amount of smut. The reasons for this are not yet definitely known.

The liquid treatments used for barley were applied also to Aurora oat for the control of loose and covered smuts. The same procedure was used as in the case of barley, and similar results were obtained, as shown in table 2. Both Germisan and Uspulun at .3 per cent strength for one hour controlled the oat smuts perfectly, while Semesan allowed only a slight trace to appear. Formalin at .3 per cent for thirty minutes eliminated the smut, but reduced the percentage of germination in the field. Colloidal sulphur and copper sulphate reduced the smut to an almost negligible amount, while Corona 640–S was worthless as a preventative for oat smuts. Soaking the seed in tap water for an hour, as in the case of barley, caused a slight increase in the amount of smut.

SUMMARY

1. Among twenty dusts used in experiments for the control of bunt, copper carbonate was found to be very satisfactory from the standpoint of germination, control, and yield, even when containing only 18 per cent of metallic copper. Its low cost and abundant supply make it the logical dust to recommend to the farmer. Several other dusts have been found equal or even superior to copper carbonate for bunt control, but their high cost or limited supply at present prevent their extensive use.

2. Germisan, Uspulun, and Semesan, in the order named, were most effective for combating covered smut of barley and the covered and loose smuts of oats. Formaldehyde controlled these smuts but caused some injury to seed.

3. Soaking oats and barley in water before sowing seems to increase the amount of smut.

OFFICE OF CEREAL INVESTIGATIONS,
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UNITED STATES DEPARTMENR OF AGRICULTURE.

THE RELATION OF SOIL TEMPERATURE AND SOIL MOISTURE TO THE DEVELOPMENT OF HEAD SMUT OF SORGHUM[1]

J. J. Christensen

With One Figure in the Text

INTRODUCTION

From field observations and experiments covering a period of years, it appeared that soil temperature and soil moisture determine to a great extent the occurrence and severity of head smut of sorghum, caused by *Sorosporium reilianum* (Kühn.) McAlpine. For this reason the writer made experiments to determine the effect of these two factors on the development of the disease.

METHODS

Soil temperature experiments were made in constant temperature tanks. The water in these tanks is heated by electric heating elements enclosed in waterproof brass containers. These are situated a few inches above the bottoms of the tanks. The temperature is regulated by thermostats devised and manufactured by the Specialty Manufacturing Company of St. Paul, Minnesota. These have proved more satisfactory than the generally used relay thermostats. They are operated by the contraction and expansion of bulbous discs containing ether, and immersed near the surface of the water in the tanks. The electrical contact is made and broken by the tilting of a sealed glass tube, containing nitrogen, a few cubic centimeters of mercury, and the electrodes.

The soil cans used for these tests were 20 cm. in diameter and 44 cm. deep. Each can contained the same quanitity of soil, the upper level of which was 3–4 cm. below the water level in the tanks, and was covered with 2–3 cm. of granulated cork.

Two different lots of soil were used. In 1924, heavily infested soil was obtained from a field at Waconia, Minnesota. In 1925, a black loam from University Farm, St. Paul, Minnesota, was used. In both trials three parts of soil were mixed with one part of sand. Smut spores and cultures of the organism were thoroughly mixed with the soil before the cans were filled.

[1] The writer is indebted to Dr. E. C. Stakman for valuable suggestions and helpful criticisms.

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In 1924, six tanks, each with four cans, were maintained at temperatures as follows: 16°, 21°, 25°, 28°, 31°, and 34° C. In 1925, seven tanks, each with six cans, were kept at the following temperatures: 12°–16°, 16°, 20°, 24°, 28°, 32°, and 36° C. In this last test, the soil in half the number of cans in each of the temperature tanks was adjusted to low and the other half to high moisture content. On an oven dry basis the moisture contents were approximately 15 and 25 per cent respectively. The moisture content of the soil in different tanks fluctuated somewhat, but in every

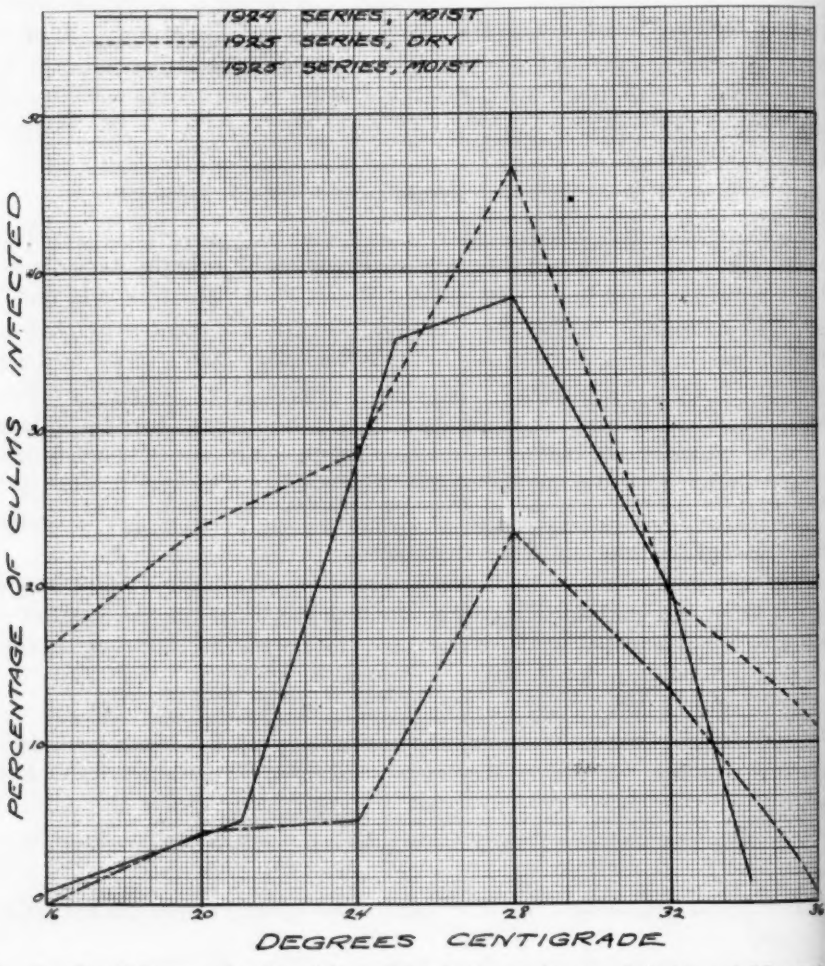

FIG. 1. The influence of soil moisture and soil temperature on infection of Minnesota Amber cane by *Sorosporium reilianum*.

case twice as much water was added to the cans in the wet series as to those in the dry series.

There was some variation at the lower temperatures: on two separate occasions the tanks kept at 16° and 20° C. varied two degrees for a few hours. The temperature of the coldest tank gradually rose from 12° to 16° C. before the completion of the three-weeks' experiment. However, the soil temperature seldom deviated more than one-half degree from those given.

The aerial parts of all the plants in a given test were exposed to similar atmospheric conditions. After the plants had developed from two to four leaves, they were transplanted to the field, where they matured. Smut counts were made in September.

Two selfed lines of Minnesota Amber sorghum, improved by Dr. J. J. Willaman of the Agricultural Biochemistry Division, University Farm, St. Paul, Minnesota, were used in all the experiments.

RESULTS

The results obtained in 1924 and in 1925 agree very closely with each other. They tend to prove that the interaction of soil moisture and soil temperature determines whether or not infection occurs, and the severity of the attack. The results are summarized in tables 1 and 2.

TABLE 1.—*The influence of soil temperature on infection of Minnesota Amber cane by Sorosporium reilianum in 1924*[a]

Temperature in degrees C.	Total number culms	Number culms infected	Per cent culms infected
16	141	1	0.71
21	57	3	5.26
25	92	33	35.87
28	73	28	38.35
31	149	29	19.46
34	107	1	0.93

[a] Moisture percentage not determined.

It is evident that *S. reilianum* can infect sorghum seedlings at temperatures ranging from 16° to 36° C. The results indicate also that the infection was not favored either by relatively high or low temparatures. From tables 1 and 2, and figure 1, it is evident that rather high soil temperature (20°–30° C.) during germination of the seed and emergence of the seedling favor infection. The highest percentage of infection was obtained at 28° C. The upper temperature limit for infection is probably near 36° C. and the lower limit apparently is not much below 16° C.

From table 2 and figure 1, it is obvious that low soil moisture (15 per cent on an oven dry basis) was much more favorable for infection than high soil moisture (25 per cent). In the moist series no smut developed at 16° C., and it was materially reduced at other temperatures. The minimum temperature for infection varies with the amount of moisture in the soil.

It is interesting to note that the thermal range for infection coincides fairly closely with that for spore germination[2] and growth of the pathogene in culture. Potter found that the optimum temperature for growth of the organism in artificial culture was between 28° and 30° C.

TABLE 2.—*The influence of soil moisture and soil temperature on infection of Minnesota Amber cane by Sorosporium reilianum in 1925*

Temperature in degrees C.	Moist series[a]			Dry series[b]		
	Total no. culms	No. culms infected	Per cent culms infected	Number plants	Total no. culms	Per cent culms infected
12–16	48	0	0	35	1	2.82
16	35	0	0	111	19	16.21
20	288	13	4.51	247	59	23.89
24	272	14	5.15	276	73	26.45
28	171	40	23.39	234	109	46.50
32	188	25	13.29	235	45	19.15
36	217	1	0.46	202	22	10.89

[a] 25 per cent moisture on oven dry basis.

[b] 15 per cent moisture on oven dry basis.

There was no correlation between the rate of germination of the sorghum seed and the percentage of smut which developed. Seeds kept at 16° C. required from 10 to 14 days to emerge, while those kept at 28° C. appeared above the surface in 3 or 4 days. Above 30° C. the time for germination was shorter than at 28° C. Seeds germinated most rapidly at 34° and 36° C., and the plants grew best at 28° to 36° C. These results seem to indicate that soil temperature and soil moisture determine the amount of smut by affecting the pathogene directly, rather than indirectly by influencing the host plant.

A low percentage of infection appears to be correlated with low temperature and high precipitation. The variation in soil moisture and soil temperature at planting time no doubt explains the differences in the severity of the disease in the same fields in different seasons. The optimum temperature for germination of spores, production of sporidia, and for infection of sorghum seedlings seldom occurs in Minnesota at planting time.

[2] Potter, Alden A. Head smut of sorghum and maize. Jour. Agr. Res. 2: 339-371. 1914.

Undoubtedly this is one of the most important factors in reducing the severity of the disease in those localities of the state where sorghum is grown intensively.

SUMMARY

1. A preliminary study was made, under controlled conditions, of the effect of soil moisture and temperature on the development of head smut of sorghum.

2. Seedlings of sorghum became infected in dry soil at temperatures ranging from 16° to 36° C. In moist soil no infection occurred at 16° C. The amount of infection was greatly reduced toward the two extremes of soil temperature.

3. The optimum soil temperature for infection was 28° C. The minimum, and probably the maximum, fluctuates with the percentage of moisture in the soil.

4. High soil moisture materially reduced the percentage of smutted plants at all temperatures and also narrowed the thermal range for infection.

5. The results indicate that soil temperature and moisture during the period of germination and emergence of sorghum plants determine to a large extent the severity of the disease under field conditions.

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FRUITLET BLACK ROT DISEASE OF PINEAPPLE

HENRY D. BARKER

WITH TWO FIGURES IN THE TEXT

During the summer of 1924 the writer's attention was called to a disease occurring in pineapples at Cap Haitien, Haiti.

Externally the diseased fruit appears to be quite normal. During the past year several thousand fruits have been cut in an intensive study of this condition. The results indicate that in certain desirable commercial varieties, such as the Smooth Cayenne, the proportion of diseased fruits may run as high as 50 to 75 per cent during certain seasons of the year. Unfortunately the disease is apparently more severe during the summer season, which is also the principal harvest season of the fruit. A second, and generally much smaller, crop of fruit is produced during the winter months, when the disease may be less severe. Available literature indicates that the disease is unknown in the pineapple region of Hawaii. Nowell[1] describes what is apparently the same disease as occuring in various parts of the West Indies, and says that the existence of this trouble has a serious reaction on attempts to establish the pineapple industry.

The most conspicuous symptoms that we have observed are briefly as follows:

A cut fruit may reveal one or many affected fruitlets in which there are dark brown or blackish spots (Fig. 1). The diseased tissue is typically dry and more or less hard. This is so marked in severe cases that diseased fruits can be distinguished as they are being cut in two. In many cases the disease appears to commence in the placentae and extend in dark, somewhat granular radiations. A portion, or all, of the inner surfaces of the individual fruitlet may be affected, or the entire fruitlet may be blackened throughout. Even in the more severe cases, it appears to be a fruitlet rot rather than a general rot of the entire fruit.

In general, the disease does not appear to affect the connective tissue. In certain cases, however, the fibrovascular bundles in the core of the fruit are distinctly browned (Fig. 1). This peculiar rot does not manifest itself in the green or immature fruit, or even in immature fruitlets sometimes found near the top of a ripe fruit. It develops during the process of ripening, but does not spread in storage or after-ripening. A diseased

[1] Nowell, W. Diseases of crop-plants in the Lesser Antilles. West India Committee, 14 Trinity Sq., London. 349–350. 1923.

fruit may be kept until yeasts and other organisms have completely fermented and decayed it, with no extension of the blackened areas. This disease must not be confused with the several somewhat similar, but less serious, fruitlet spots or rots that occasionally occur in pineapples. The

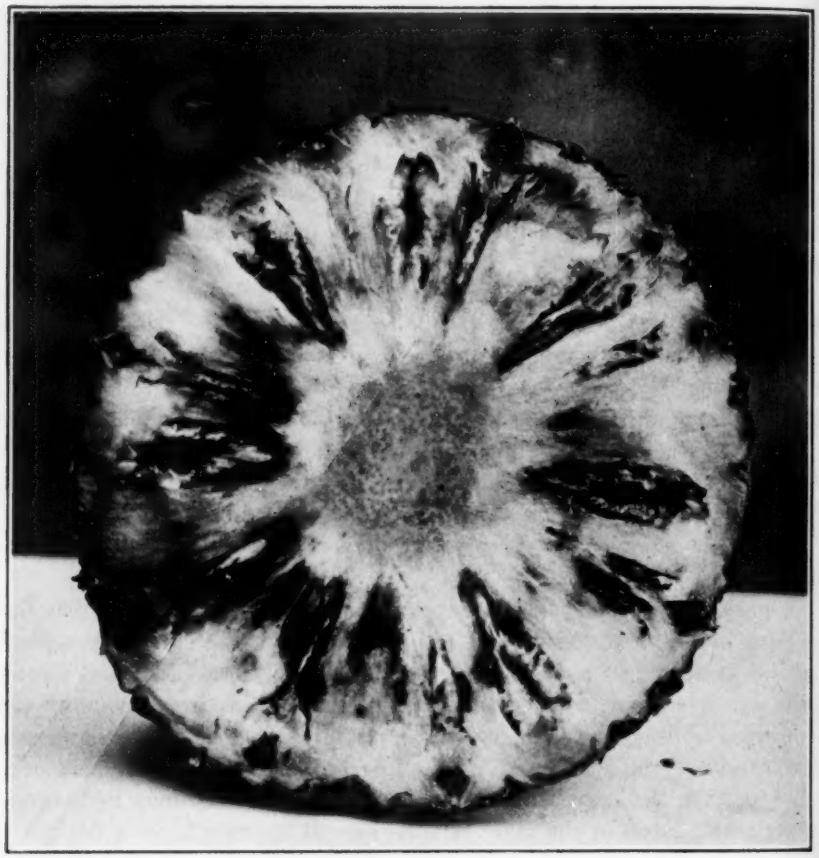

FIG. 1. Photograph of cross-section of fruit affected by fruitlet black rot. Natural infection, unusually severe. Note blackened fibrovascular bundles.

evidence indicates that unusually large fruits are more subject to attack than are the smaller ones. No external symptoms of the disease have been discovered. However, in a known, but apparently undescribed, disease, a "gummosis," in which amber-like gum or waxy secretion is exuded from the eye, seems to be a related phenomenon.

Under natural conditions in the field here, gummosis is rare, and the fruitlet underneath the gummed eye may or may not be blackened. How-

ever, when the fruitlet black rot is produced artificially by needle puncture, gummosis is a quite commonly associated symptom. This possibly may be accounted for by the fact that, under natural conditions, there is no apparent connection between the affected part of the fruitlet and the external surface of the fruit. The greater amount of inoculum and the greater injury to the tissues during artificial inoculation also may be important factors.

Although the disease has occurred in the West Indies for some 20 years, apparently very little is known concerning its actual cause and nature. From the work of Howard, in 1901, and of other mycologists of the Imperial Department of Agriculture of the West Indies, Nowell concludes: "A species of *Penicillium* is uniformly found to occupy the spots, fruiting in small cracks and in the cavity of the ovary.

"The beginning of the spots, and the entry of the fungus into the tissues is associated, commonly at least, with perforations in the tough wall of the old floral chamber (the eye) near the insertion of the style. Species of *Penicillium* are generally present on the withered floral organs, and it is regarded as probable that the fungus depends for access to the pulp, on punctures made by insects or mites inhabiting the enclosed floral chamber."

Under conditions in Haiti *Penicillium* also has been found to be almost universally present on the old withered floral organs in the "eye," in both diseased and healthy fruits. However, within the tissue of the diseased fruit *Penicillium* is rarely found, except in the more advanced stages, when it and a great variety of secondary filamentous fungi, yeasts, and bacteria become common.

In plates made from the earliest stage of the disease, the organism most commonly obtained was a pale yellowish bacterium with rugose colonies. Inoculations of this organism into the fruitlets, by needle punctures, were made in the field on fruits that were judged to be about 10 days from maturity. The first inoculations were made through the floral chamber or eye. These were then sealed with grafting wax. Practically 100 per cent infection was obtained both in the inoculated fruits and in the checks which had been punctured and sealed. In the second series of inoculations the surface of individual fruitlets was disinfected with 1–1000 bichloride of mercury, punctures were made from the outside and immediately sealed with grafting wax. Care was exercised to prevent the needle from entering or puncturing the floral cavity. This method gave uniform infection in the inoculated fruits, while the checks remained healthy, that is, the percentage of diseased fruits in the checks was no greater than the average field run at that particular time. Furthermore, the diseased fruitlets occurring in the checks were distributed throughout the fruit in such a way as to suggest no relation to punctured fruitlets. The artificially produced

black fruitlet rot was typical, and characteristic of the naturally produced disease (Fig. 2). The only divergence noted was the associated "gummosis" in the case of the artificially produced disease. In some cases the disease seemed to be strictly localized, being confined to the inoculated fruitlets or sometimes spreading to a few contiguous fruitlets; in other cases it seemed to be more or less systemic, spreading to all the ripe fruitlets and causing a browning of the vascular strands in the core of the fruit. This apparent difference in reaction has not yet been explained. Reisolations of the same yellow bacterial organism were obtained from the typically blackened tissue, from the browned bundles in the core, and from the gummy exudate.

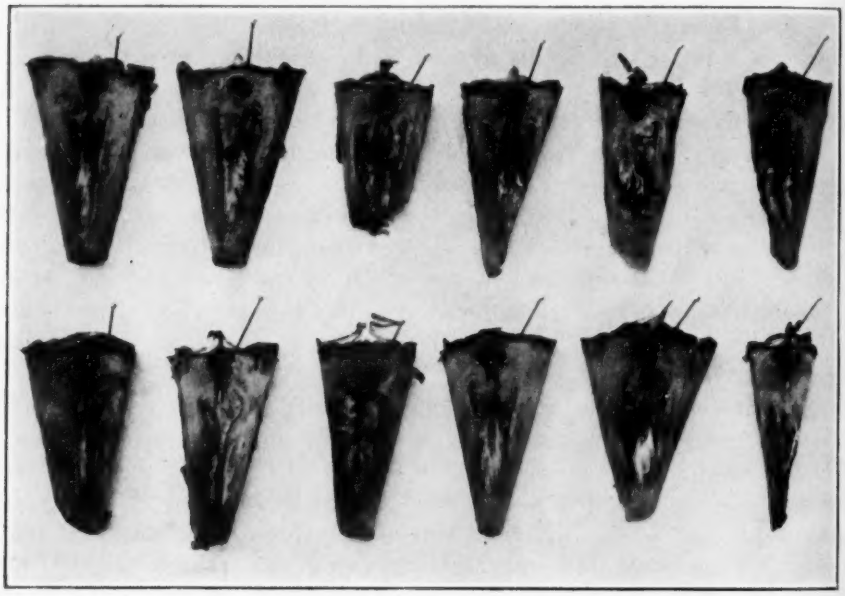

FIG. 2. Early stages in the disease. Individual fruitlets cut from cross-section of fruit in which disease was produced artificially. Note the beginning of the disease in the placentae and its development in the fruitlet.

For many reasons the problem has been, and in many features still is, a very perplexing one. Upon the completion of experiments now in progress details regarding the identity of the causal organism, attempted control measures, etc., will be published in a joint paper with Dr. G. N. Wolcott, Entomologist of the Haitian Department of Agriculture.

SUMMARY

1. A fruitlet black rot disease of pineapples occurring in Haiti is described. The disease is very severe in the improved variety Smooth Cayenne, in which 50 to 75 per cent of the fruits may be affected.

2. Cultures made from tissues in the earliest stage of the disease yielded commonly a pale yellowish bacterium with rugose colonies.

3. Inoculations with this organism produced typical symptoms of the disease, and the organism was reisolated from the inoculated tissues.

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PERONOSPORA PARASITICA (PERS.) DE BY ATTACKING CABBAGE HEADS

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WITH ONE FIGURE IN THE TEXT

The attack of full-grown cabbage heads by *Peronospora parasitica* has not been mentioned in the literature. The general opinion is that the fungus attacks only very young plants.

The fungus not only attacks the outer leaves of the full-grown cabbage heads but also penetrates into the inner leaves, the mycelium being able to pass from one leaf to the other. In the cabbage-growing districts of Holland the fungus can easily be found throughout the year.

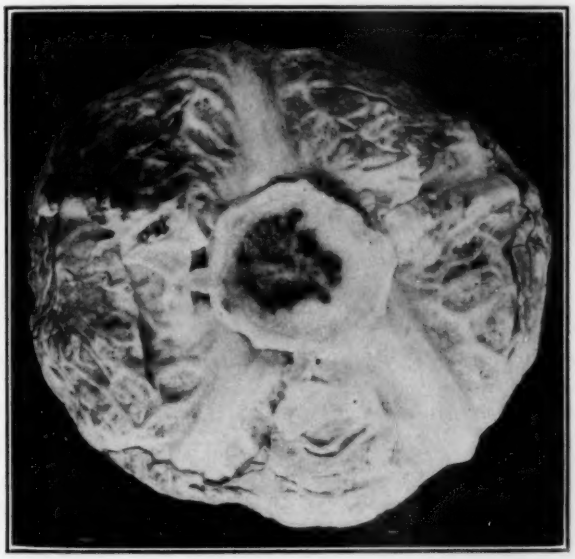

FIG. 1. Cabbage head attacked by the mycelium of *Peronospora parasitica* (Pers.) de By: the conidiophores are visible.

The old yellow leaves nearest to the ground usually are covered with irregular, circular bordered, black spots, in which the mycelium lives and on which the conidiophores are to be found.

As stated in the literature, overwintering can take place by means of oospores. Observations during the winter 1924–25, however, showed that the mycelium also can live throughout the winter in cabbage leaves, and that exposure to frost does not kill it.

Conidia may germinate and the mycelium may spread in infected cabbages stored during the winter. The longer the cabbage is stored, the more severely it is likely to be injured. The fungus even may penetrate into the parenchyma of the stem when cabbage has been stored for a long time.

The author of the present note agrees with Gäumann[1] that the *Peronospora* of *Capsella bursa pastoris* is another strain, which does not attack cabbage.

Control measures: removal of *Capsella bursa pastoris*, on which *Peronospora parasitica* also lives (mentioned as a means of control by Sorauer[2]), is not necessary. When weeding and hoeing the fields, one might at the same time gather and destroy those lower leaves of the cabbage plants which are covered with the fungus. The white cabbage varieties are more susceptible than the red ones.

Details will be published later.

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[1] Gäumann, E. Über die Formen der *Peronospora parasitica* (Pers.) Fries. Beih. Bot. Centralblatt. 35: 395–533. 1918.

[2] Sorauer, P. Handbuch der Pflanzenkrankheiten 2. Vierte Auflage, S. 213.

RHIZOCTONIA CROWN ROT OF CARROTS[1]

R. P. WHITE

WITH ONE FIGURE IN THE TEXT

Rhizoctonia crown rot of carrots was first observed in Kansas in June, 1924, in the Kaw River Bottoms immediately west of Kansas City, Kansas. Excessive rain had fallen for two weeks preceding the discovery of the trouble, bringing about saturated soil conditions and high humidity around the bases of the plants.

Dr. Charles Chupp states in correspondence[2] that this disease is quite prevalent in New York State and occasionally does considerable damage, being most injurious in localities where young carrots are sold with the tops still attached. The *Corticium* stage, he states, appears as a white weft about the bases of the leaves, almost wherever carrots are grown. This condition never had been observed in Kansas, however, until June, 1924.

The carrots affected were apparently stunted, but upon close observation this apparent stunting was found to be due to the decay of the bases of the central leaves, which either failed to develop normally or were rotted off completely. Older leaves, that were still attached to the root but were partially decayed or only slightly infected, had red leaf margins. It is questionable whether this red and bronze discoloration of the leaves is specifically associated with the infection of *Rhizoctonia* on carrots. This symptom did not appear on artificially inoculated plants in the greenhouse.

In badly diseased specimens a crown rot of the fleshy root was evident as a soft brown decay entering the crown and forming an inverted cone-shaped mass of decayed tissue. In such plants all of the central leaves had been rotted off, leaving only a few of the older leaves around the edge of the decayed zone.

Isolations were made from leaf bases and decayed crowns. The only organism constantly associated with the lesions was a species of *Rhizoctonia*, similar in gross and microscopic characters to *Rhizoctonia solani*. No cross inoculations were made to potato or other hosts. Secondary organisms were abundant, especially in the diseased roots, but none was proved pathogenic.

Both young and nearly mature carrot plants were inoculated in the crown with a small block of agar containing vigorously growing hyphal tips

[1] Paper No. 248 of the Department of Botany and Plant Pathology, Kansas Agricultural Experiment Station.

[2] Correspondence dated October 12 and October 26, 1925.

of *Rhizoctonia*. Inoculations at first were not very successful owing to the fact, as it was found later, that relatively high temperatures and high humidity are required for infection. Infection results with plants inoculated and kept in a damp chamber for two days were inconsistent, while similarly inoculated plants never failed to develop the typical brown decay of the leaf bases and rot of the crown if left in the damp chamber for seven days (fig. 1). The typical weft of white mycelium developed around the bases of the leaves.

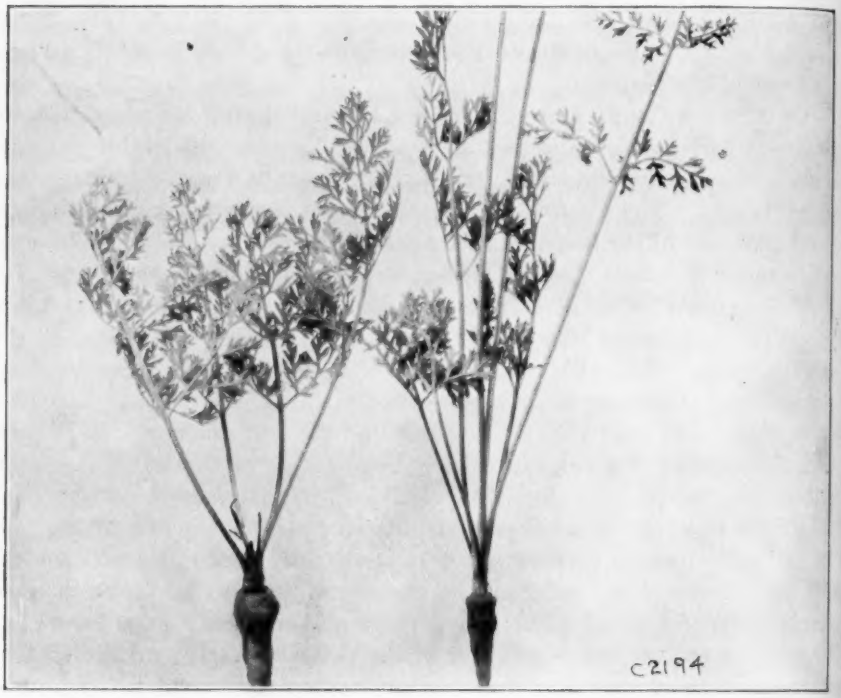

FIG. 1. Crown rot of carrots. Healthy plant on right: plant on left artificially inoculated with *Rhizoctonia sp.*

Rhizoctonia sp. is evidently a weak parasite on carrots, infecting at the crown only under conditions of high humidity. It seems as if this trouble could be avoided in wet seasons, by wider spacing of the rows to allow better circulation of the air between the plants, although no experiments upon control measures were attempted. No loss should be expected in Kansas in dry seasons or in seasons of normal precipitation.

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PHYTOPATHOLOGICAL NOTES

Physiologic forms of Colletotrichum lindemuthianum (Sac. et Mag.) Bri. et Cav. in the Netherlands.[1]

In the European literature on the bean anthracnose, statements can be found which indicate that certain bean varieties are immune from the disease in one place and not in another.

This fact led to the conjecture that in Europe there might be physiologic forms of the pathogene similar to those which have been found in America.

Because it was desired to compare the physiologic forms eventually to be found with those found by American investigators, the infection experiments were made with the same bean varieties used by Barrus and Burkholder of Cornell University and by Leach of the University of Minnesota, who kindly forwarded, at our request, the seed for the experiments.

Five physiologic forms have been found to date which are different from those of Barrus, Burkholder, and Leach. Two forms, which gave the same reaction on the seven bean varieties of Leach's key, and which therefore were indistinguishable by it, differed in pathogenicity for other bean varieties.

Four forms were isolated from diseased pods of *Phaseolus vulgaris* L. from different parts of the Netherlands, and one form from a pod of *Phaseolus multiflorus* Wild. The last form is characterized by its extremely rapid growth on nutrient agars.

The vulgaris forms differed in pathogenicity and in growth and appearance of the colonies on nutrient agars. All produced the ascogenous stage in the laboratory; the multiflorus form has not yet been investigated in this respect.

The experiments will be continued, and will be described *in extenso* in a later publication.—H. R. A. MULLER, Institute for Phytopathology, Laboratory for Mycology and Potato Research, Wageningen, Holland.

Banana Wilt Disease on the Island of Singapore.—Dr. Otto A. Reinking, of the United Fruit Company, writes from Singapore as follows:

"The wilt of bananas has been found on the island of Singapore in the outskirts of the city of Singapore. How widespread the disease is has not been determined. External and internal symptoms show the disease to be typical of the Caribbean banana wilt due to *Fusarium cubense*. Microsco-

[1] Received for publication September 28, 1925.

pic examination shows the presence of typical mycelial strands in the xylem tissue. The disease in all probability is due to the same *Fusarium* as is present in Central America, but this can not be stated definitely until cultures are obtained. Cultures of the organism will be made at the earliest opportunity. The disease so far has been definitely observed in the Philippines and Singapore. The disease was observed as such for the first time in Singapore on October 13, 1925.''—JOHN R. JOHNSTON, United Fruit Co., Boston, Mass.